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## Scientific Note

# Spatial choice is biased by chemical cues from conspecifics in the speckeled worm eel *Myrophis punctatus*

Rodrigo Egydio Barreto[1,2], Maria Fernanda Coelho Junqueira[3], Tan Tjui-Yeuw[3] and Gilson Luiz Volpato[1,2]

The speckeld worm eel *Myrophis punctatus* lives in high-densities assemblages, and usually digs through, or lies on the substrate. These behaviours could lead to chemical marks on the substrate and could modulate the spatial distribution in this species. We tested the hypothesis that the spatial choice of the speckled worm eel is modulated by the presence of conspecific odour on the substrate. Here, we showed that the speckled worm eel avoids the substrate area containing the conspecific odour, indicating that this chemical cue modulates the eel's spatial decision. The eels clearly detected the conspecific's odour. This perception might indicate the presence of conspecifics into the substrate. Since the eels avoided an area containing conspecific odour, we suggest this may be a response that avoids the consequences of invading a resident-animal's territory.

A enguia mirongo-mirim *Myrophis punctatus* vive em agrupamentos de alta densidade populacional e comumente se enterra ou permanece sob o substrato. Esses comportamentos podem levar a marcas químicas no subtrato e podem, portanto, modular o uso do espaço nessa espécie. Neste estudo, testamos a hipótese de que a preferência espacial da enguia mirongo-mirim é influenciada pela presença de odor do animal coespecífico no subtrato. Mostramos que as enguias evitam a área que contém tal odor, indicando que as decisões de ocupação espacial podem ser influenciadas por pistas químicas de coespecíficos. As enguias claramente detectaram o odor de um animal coespecífico e essa percepção poderia ser um indicativo da presença de um coespecífico enterrado no substrato. Visto que elas evitam uma área contendo tal odor, sugerimos que isso poderia ser uma resposta para evitar invadir o território de um animal residente.

**Key words:** Chemical communication, Chemoreception, Estuarine fish, Territoriality, Aggression.

Vision plays an important role in dealing with environmental challenges in fish, such as predator recognition (Barreto *et al*., 2003; Freitas & Volpato, 2008) and social communication (Oliveira *et al*., 2001). On the other hand, when inhabiting turbid water where visibility is low, fish may use chemical cues to interact with other fish (Wisenden, 2000).

During conspecific communication, olfaction is used to obtain information about social status (Giaquinto & Volpato 1997; Gonçalves-de-Freitas *et al*., 2008) and distinguish between kin and non-kin fish (Brown & Brown, 1993). Moreover, the level of aggression can be modulated by non-related individuals' chemical cues (Griffiths & Armstrong, 2000).

The speckled worm eel (*Myrophis punctatus* Lütken, 1852) inhabits rocky coasts, coral reefs, and mainly estuaries, a turbid water environment. This species spends most of its time motionless or digging through the sand substrate. During low tide, they inhabit crab holes avoiding desiccation until the next flood tide (Barletta *et al*., 2000). According to these authors, they usually live in areas with a high density of individuals. These conditions (high density groups, turbid water and burrow into substrate) were likely selective pressures for this species, suggesting that olfactory cues should be important in conspecific interactions, including spatial use among individuals. Thus, we predict that this eel species uses chemical cues during spatial

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[1] Research Center on Animal Welfare (RECAW) and Centro de Aquicultura da UNESP (CAUNESP). rebarreto@yahoo.com
[2] Departamento de Fisiologia, Instituto de Biociências, CAUNESP, UNESP. Rubião Jr. s/n, 18618-970 Botucatu, São Paulo, Brazil.
[3] Campus Experimental do Litoral Paulista, UNESP, Unidade São Vicente. Praça Infante D. Henrique s/n, 11330-900 São Vicente, São Paulo, Brazil.

choosing. In this study, we tested this hypothesis in the laboratory, evaluating the spatial choice of this eel species in the presence of conspecific odour on the substrate.

In this context, we propose two possibilities. Firstly, eel avoids the substrate area with a conspecific odour. An area with conspecific odour should indicate the territory of another animal, and to stay in it, or to invade a conspecific area could induce fighting. In a contest, the intruder animal is usually the loser (Beaugrand & Zayan, 1985; Fayed *et al*., 2008; Morishita *et al*., 2009; Kadry & Barreto, 2010) and might experience the harmful effects of losing a fight (stress by receiving several bites, in the case of fishes, for instance see Barreto & Volpato 2006a, 2006b). Thus, it would be plausible that chemical cues are used to avoid unnecessary aggressive encounters in this eel. The second possibility comprises in the use of chemical cues for aggregating behaviour. In fact, grouping behaviour has been reported in an Anguillidae eel, the glass eel *Anguilla anguilla*, but the gregarious swimming behaviour lasted few weeks only, and it is replaced by intense aggressive interaction among individuals (Bardonnet *et al*., 2005).

Speckled worm eels (*Myrophis punctatus*; Actinopterygii; Anguilliformes; Ophichthidae) were collected on River Itanhaem's estuary (24.190°S 46.795°N). We reared a stock population (0.4 fish/L) in plastic tanks containing a constant aeration and a room temperature (24-28°C) with a half day photoperiod (12 h:12 h L/D). Fish (mean ± SD) were 58 ± 20 g and 23 ± 32 cm. Fresh shrimp or fish were offered three times a week.

The strategy of this study was conducting choice tests to assess the spatial distribution of this eel species in the presence or not of conspecific odour on the substrate. Exterior lines above the experimental tanks (70 x 30 x 45 cm) divided them into 7 equally-sized rectangle areas numbered from 1 to 7. An experimental apparatus scheme is showed on Fig. 1. Fish presence in these tank areas where quantified to indicate spatial choice. The odour was applied by keeping a conspecific confined in a plastic container in randomly chosen area (area 2 in this case) of the experimental tank for 24h. This area remained the same for all tested fish in their respective tank. This procedure, although it did not prevent any conspecific scent spread through tank water, allowed a specific substrate area to be chemically marked. After that, the odour donor and the plastic container were removed and a focus fish from the stock tank was gently introduced into the centre of the experimental tank. Next, we registered the area where the focus fish was located every minute during a 20-min period. As a control condition, we conducted the same procedure, but without any conspecific odour. We tested six eel in each odour condition.

Experimental tank was supplied with 6.3 $cm^3$ of a sand beach substrate (sediment granulometry = from 0.062 to 0.125 mm), a 10-cm water column with constant aeration through a tubing connected to an air pump and an air stone in the centre of the tank. The sand beach was treated with sodium hypochlorite (5 ml of pure sodium hypochlorite/35 l of water), rinsed with abundant tap water and dechlorinated by using Aquasafe®. No significant chlorine residual was detected, as checked by using Alcon Labcon Chlorine test®.

The frequency of fish presence among the tank areas was compared within each odour condition by using Friedman ANOVA test and post-hoc compared by Newman-Keuls test of sum of ranks (Zar, 1999). We compared data from area 2 (the randomly chosen focus area) between odour conditions by using Mann-Whitney U test. Statistical differences was set at $\alpha = 0.05$.

The Friedman ANOVA test revealed statistical differences among areas in both control (p = 0.00088) and conspecific odour (p = 0.03392) conditions. The pattern of area use, however, differed among conditions. In the control condition, we found that the eels presence in the extremes of the tank areas (area 1 or 7) were statistically similar between each other and higher than the other five central areas (area 2-6); these, in turn, were statistically similar among themselves (Fig. 2). The presence of conspecific odour on area 2 changed this pattern (Fig. 2): the presence in area 7 was statistically higher than all areas, except for area 1; in turn, area 1 was also similar to area 5, but higher than the remaining areas; area 2 was lower than all areas, except for area 3; this last area was statistically similar to area 4 and 6 but lower than area 5; these last three areas were statistically similar to each other. It was also found that the presence of eels in the areas 2 in the tank-containing conspecific odour was statistically lower than the same area in the control condition (p = 0.01632; Fig. 3).

Here we showed that the speckled worm eel avoids areas with substrate containing conspecific odour, indicating that this chemical cue modulates the eels' spatial decision. Eels commonly stayed on the substrate or swam thigmotactically. In fact, thigmotropism has been reported in fish; for instance, the swordtail *Xiphophorus helleri* (Anken *et al*., 2000) and the zebrafish *Danio rerio* (Maximino *et al*., 2010). Possibly, the use of tactile information for locomotion led to a strong natural

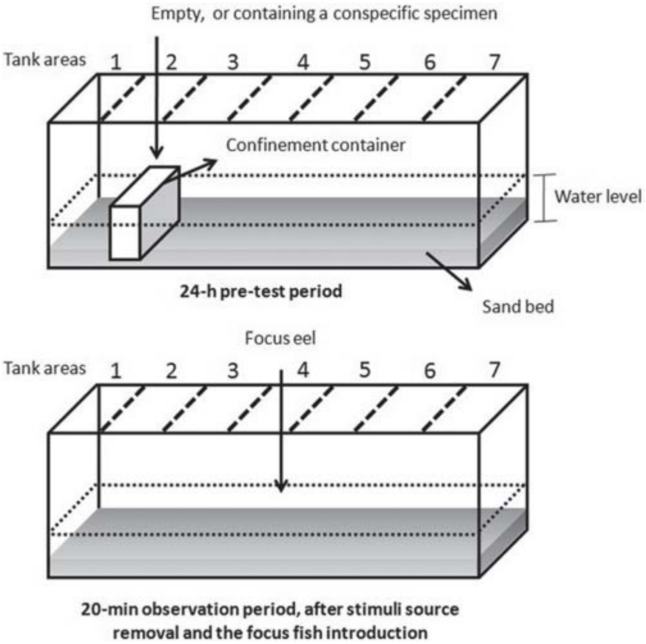

**Fig. 1.** Scheme of experimental apparatus during substrate odour labelling and eel' spatial preference testing.

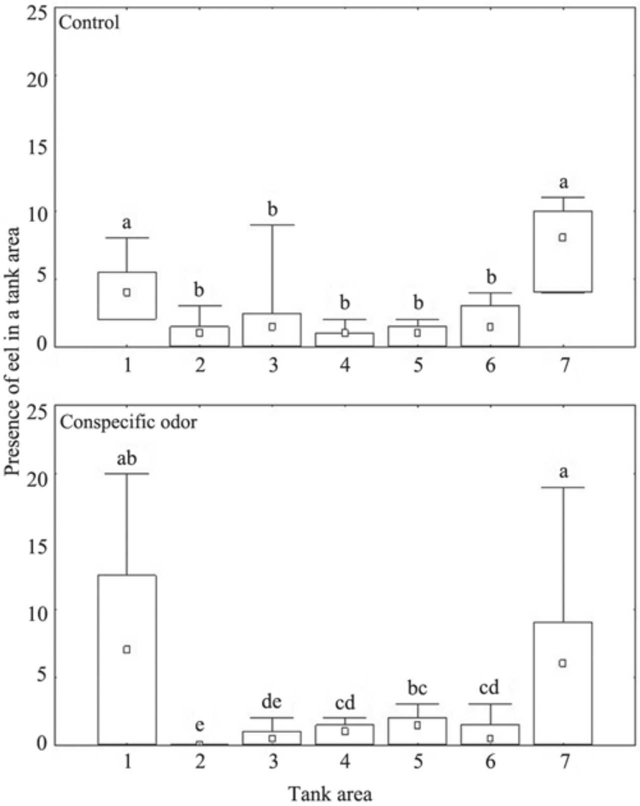

**Fig. 2.** The effects of conspecific odour on spatial choice in the speckled worm eel *Myrophis punctatus*. Data are shown as median (small square), minimum and maximum obtained value (lines) and quartiles 25-75% (boxes). The basic strategy of this study was to test eel spatial choice in the presence (Conspecific odour) or not (Control) of conspecific odour on the substrate. Median values that do not share a same letter are statistically different (Friedman ANOVA; $p < 0.05$).

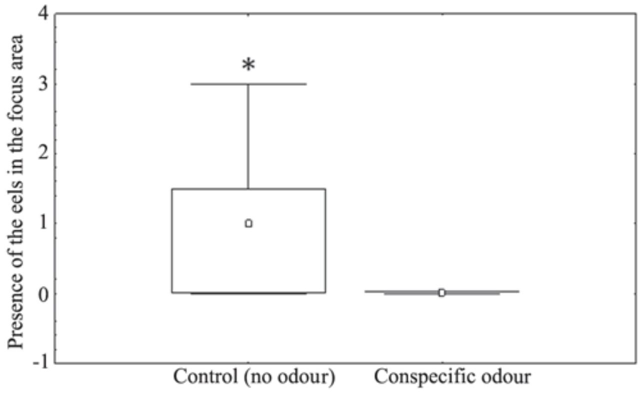

**Fig. 3.** The effects of conspecific odour on spatial choice in the speckled worm eel *Myrophis punctatus*. Data are shown as median (small square), minimum and maximum obtained value (lines) and quartiles 25-75% (boxes). In this analysis, presence of eels on the randomly chosen focus area was compared by Mann-Whitney U test and * indicates statistical difference ($p < 0.05$).

preference for the tank extreme areas due to the higher contact to tank walls. The presence of the conspecific chemical cues in the substrate did not change the choice for the tank extremities, but this odour changed the choice among the middle areas with clear avoidance for the area containing conspecific odour.

The eels clearly detected the conspecific's odour, a cue that might indicate the presence of conspecifics in the substrate. As the eels avoided an area containing conspecific odour, we suggest this behaviour happens to avoid invasion and/or staying in an owned territory (the focus fish was the 'intruder' in the tank, and the conspecific chemical cue should be assumed as the presence of a territory owner burrowed into the sand bed). Territory owners have advantages in a fight and usually win the contest (Beaugrand & Zayan, 1985; Fayed *et al*., 2008; Morishita *et al*., 2009; Kadry & Barreto, 2010). Thus, conspecific odour perception may prevent intruder animals to be stalwartly attacked, injured or prevent unnecessary use of energy in a disadvantageous dispute, thus a clearly adaptive response.

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