**Within-shoal phenotypic homogeneity affects shoaling preference in a killifish**

**Cattelan Silvia and Griggio Matteo**

**Supplementary materials**

*Fish maintenance*

After collection, fish were immediately transported to the laboratory using aerated tanks. At the laboratory fish were maintained in 60 x 40 x 35 cm glass tanks supplied with natural gravel, artificial algae, and air stones for oxygenation. Light was provided by fluorescent lamps with a 12-h light / 12-h dark photoperiod. Fish were fed fresh mussels each day. The experiment began 20 days after the fish were transported to the laboratory. At the end of the experiment, fish were released into their natural habitat.

*Binary preference test*

The experimental tank was a glass tank (60 x 40 x 35 cm, 15 cm filled with tap water) provided with natural light-grey gravel and illuminated by a fluorescent lamp. To minimise external disturbance, the walls of the experimental tank were covered with green plastic and surrounded by dark-green curtains. At the two ends of the experimental tank we positioned two transparent semi-cylinders (10 cm radius, 15 cm high).

The semi-cylinders were punctured to allow visual and olfactory communication. Before each trial, we placed the homogeneous and the non-homogeneous group in the two semi-cylinders (see Fig. 1S). The left/right location of the homogeneous/non-homogeneous stimuli was alternated between trials to avoid bias. After 4 minutes of habituation of the stimuli to their compartments, we released the focal fish in the proximity of an artificial algae positioned at the centre of the tank. The algae provided a refuge zone to the focal fish during habituation to the tank. We started to video-record when the focal fish was released in the tank and for the subsequent 15 minutes. We excluded those trials in which fish did not leave the refuge zone within 4 minutes (n = 2 trials).

*Analysis of stimuli behaviour*

For each trial, we assessed the behaviour of both stimulus shoals in term of shoal cohesion and shoal activity.

*(a) Cohesion —* We measured shoal cohesion by sampling the relative position of the three fish in the shoal every 30 seconds. We then assigned a cohesion score to these observations: ‘1’, if all subjects were within one body length from each other; ‘2’, if two subjects were within one body length from each other; and ‘3’ if the distance among the three subjects was greater than one body length. For each cohesion score separately, we performed a Wilcoxon signed-rank test. We found no difference between homogeneous and non-homogeneous groups in either cohesion score (all fish close to each other: V = 63, *p* = 0.527; two fish close to each other: V = 102, *p* = 0.277; none close to each other: V = 138, *p* = 0.223).

*(b) Activity —* We recorded shoal activity measuring the time spent by each stimulus shoal in 1- swimming in the water column, 2- swimming at the bottom of the tank, and 3- in staying motionless. After calculating the proportion of time spent in each type of shoal activity, we performed a paired t-test for each type of shoal activity separately between homogeneous and non-homogeneous groups. We found no difference between homogeneous and non-homogeneous groups in either type of shoal activity (1- swimming in the water column: t1,23= -0.489, *p* = 0.630; 2- swimming at the bottom: t1,23= -0.018, *p* = 0.985; 3- staying motionless: t1,23= 0.956, *p* = 0.349).

*Analysis of bar area*

In order to control for differences in the total area covered by bars among individuals characterized by the same number of bars, we analysed digital images of females from another experiment. We randomly selected a subsample of females (n = 10) characterized by the same number of bars (n = 9) for which we measured the body area (mm2) and the total area covered by the bars (mm2) using Image J software (<http://rsbweb.nih.gov/ij/download.html>). Then, we calculated the coefficient of variation (standard deviation / mean) on the relative area covered by bars (absolute bar area / body area). We found a small coefficient of variation for bar area (12.08%), as expected for morphological traits (e.g. [1, 2]).

[1] Blanck, A. & Lamouroux, N. 2006 Large-scale intraspecific variation in life-history traits of European freshwater fish. *Journal of Biogeography* **34**, 862-875. (doi:10.1111/j.1365-2699.2006.01654.x).

[2] Pomiankowski, A. & Moller, A.P. 1995 A Resolution of the Lek Paradox. *P R Soc B* **260**, 21-29.