Does the presence of conspecifics facilitate exploratory behaviour in a cichlid fish (*Etroplus suratensis*)?

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Abstract

Animals confronted with any kind of novelty show behavioural responses driven by avoidance and exploration. The expression of both tendencies is modulated by anxiety. Especially in group-living animals, the presence of conspecifics can reduce anxiety in novel situations and hence increase exploratory tendency. Such intensified behavioural responses triggered by the social environment of an individual are called social facilitation. Here, we tested for social facilitation on the exploratory tendency of group-living juvenile Green chromide cichlid fish (*Etroplus suratensis*) by assessing each individual twice in an open field test: once alone and once together with a conspecific. Contrary to our expectations, we found no difference in exploratory behaviour between the groups. However, our results suggest that changes in exploratory tendency across the two treatments were highly individual, both in extent and direction, and are likely driven by the presence of the conspecific and the focal individual’s own behavioural type.

Keywords

social facilitation, open field test, cichlids, co-action effect, animal personality.

1. Introduction

The behavioural response of an individual towards any kind of challenge or stress can be influenced by the presence of conspecifics, an effect that is called social facilitation. Experiments on social facilitation originated in the field of human psychology and date back to the end of the 19th century (Triplett, 1898). Depending on the complexity of the task, the behavioural
performance of a focal individual can improve or suffer from the presence of conspecifics (Zajonc, 1965). The concept of social facilitation is very broad and includes the definition of social learning, a term that describes an (often adaptive) form of learning during which an individual makes use of social instead of private information. Here, changes in behaviour are often due to passive spectators, with the most prominent examples including copying behaviour and audience effects (Heyes & Galef, 1996; Laland, 2004; Hoppitt & Laland, 2013). The definition of social facilitation also includes behaviours that occur in the presence of other individuals also engaged in the same activity (Zajonc, 1965). The two terms social learning and social facilitation thus do not reflect different concepts. Instead, social facilitation covers a broader range of observations.

Early studies on social facilitation in non-human animals were strongly biased towards feeding behaviour in mammals (e.g., Harlow, 1932; Harlow & Yudin, 1933; James, 1953; Shelley, 1965). Later, also other classes of vertebrates were observed to show clear signs of social facilitation in various contexts. Especially group-living animals such as swarm fish provide excellent study systems for the investigation of this social phenomenon (Ryer & Olla, 1991).

An important function of schooling in fish is that it enables social facilitation, which in turn may lead to a better performance of individuals in various situations, ultimately increasing the chances of survival. For instance, spatial challenges were learned more quickly by goldfish (Carassius auratus) when kept in groups than when being isolated (Welty, 1934). Also, learning through classical conditioning can be improved in the presence of conspecifics (e.g., Zion et al., 2007). Furthermore, social facilitation in mixed-size school forming Red Sea pomacentrids (Dascyllus marginatus) was shown to lead to higher rates of survival through better recognition and avoidance of predators (Karplus et al., 2006).

In the current study, we used the Green chromide (Etroplus suratensis), a freshwater cichlid fish with strong group cohesion, to ask the question of whether exploration of a novel environment is more efficient in the presence of conspecifics. An animal confronted with any kind of novelty shows behavioural responses that are driven by two antagonistic tendencies: the avoidance of novelty (i.e., neophobia) and the curious exploration of novel situations. The relative expression of these conflicting tendencies is considered to be modulated by anxiety (Galhardo et al., 2012). We expected that the
presence of a conspecific would reduce levels of anxiety in the Green chromide, which would result in an elevated readiness to explore a given environment. We used the well-established open field test to assess exploratory tendency of our test fish (Gould et al., 2009; Stewart et al., 2012). During this test, an individual (or a group of individuals) is placed into a novel environment without hiding opportunities and is free to explore as much of the given area as it decides to within a given amount of time. Each individual was tested twice in the same experimental setup, once alone and once in the presence of a conspecific, in randomized order and with one week in between the tests. To avoid dominance effects or any kind of sexual interactions, we exclusively used subadult females of the same size and age.

We hypothesized that fish in the group treatment would (i) explore a higher percentage of the test arena, and (ii) show less thigmotaxis (‘wall hugging’, an indicator for anxiety: Schnörr et al., 2012) than the same individuals in the single treatment.

2. Material and methods

2.1. Experimental animals and maintenance

Test fish were lab-born *E. suratensis*, a cichlid species that inhabits fresh and brackish waters in parts of India. Subadult fish show strong group cohesion and are, therefore, excellent test subjects to investigate the influence of conspecific presence on behavioural responses.

Offspring born in January 2020 (approx. 50 individuals) was maintained in an aerated and filtered 250-l mixed-sex stock tank at 25°C under a 12/12 h light/dark cycle (lightdec LED: 6000 K, 1300 lumen, 30 Hz) at the University of Basel, Basel, Switzerland. The tank was equipped with different shapes of clay shards as hiding opportunities. Fish were fed once a day ad libitum with frozen Artemia, shrimps, mosquito larvae and/or spinach. To maintain water quality, half of the tank water was replaced by tap water once a week.

For our experiments, conducted in March 2021, we randomly chose \(N = 20\) subadult female individuals. All fish stemmed from the same clutch (14 months old), and were of similar standard length (mean ± SD: 5.02 ± 0.33 cm; Figure 1). We marked the test fish with visible implant elastomers (VIE, Northwest Marine Technology, Anacortes, WA, USA) under the dorsal epidermis, following the protocol described by Croft et al. (2003). This unique colour tag allowed us to identify individuals throughout the
behavioural experiments. All experimental treatments were performed under permit No. 2356 issued by the cantonal veterinary office Basel-Stadt.

2.2. General testing procedure

All fish were assessed twice for their exploratory behaviour with one week in between the tests. In one of the assessments, the focal fish was tested alone (single treatment), while in the other assessment it was tested together with a conspecific (group treatment; see Table 1 for group assignments). The order of treatments was randomized.

In between the behavioural assessments, all test fish were held in individual glass tanks (25 × 25 × 22 cm), allowing visual contact to at least two neighbouring test fish. The use of individual tanks minimized handling time and ensured fast and accurate individual identification.

After the second behavioural assessment, test fish underwent a standard procedure during which they were photographed and weighed upon a brief anaesthesia (Figure 1). None of the test fish showed any signs of distress.
Table 1.
The order of treatments (single vs. group) was randomized across the 20 test fish.

<table>
<thead>
<tr>
<th>Focal fish ID</th>
<th>Treatment 1 (= week 1)</th>
<th>Treatment 2 (= week 2)</th>
<th>Conspecific ID</th>
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<td>Group</td>
<td>Single</td>
<td>19</td>
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</table>

The table shows which individuals were randomly matched with one another for the group treatment. The maximum size difference (standard length) between individuals of the same group was 5 mm.

after this procedure and all fish accepted food flakes straight away. After an additional day of resting in their individual tanks, all fish were returned to their original maintenance tank.

2.3. Assessing exploration: open-field test

In the open-field test, the focal individual is placed into a test tank without hiding opportunities, in which its movement patterns are analysed for a certain amount of time. Two common variables are measured in this context: (i) area explored (Stewart et al., 2012) and (ii) thigmotaxis (wall hugging; Schnörr et al., 2012). The first variable describes how much of the total tank area has been visited by the test individual and is often used as a proxy for exploration tendency. Thigmotaxis refers to the animal’s tendency to swim close to the walls versus swimming in the central area of the test tank, which
can serve as an additional measurement of fear with high thigmotaxis reflecting a higher level of fear.

The open-field test tank was equipped with sand on the bottom and all inside walls were covered with matt grey plastic foil to reduce visual distraction by the lab environment and reflections of the test fish in the glass walls of the tank. With hand net(s), we gently transferred one (single treatment) or two (group treatment) test fish(es) into one end of the test tank (starting position). This mild handling stress elicited a short freezing time in most of our test fish, i.e., the fish remained motionless in the same spot, which is known to be a common predator-avoidance behaviour (Brown & Godin, 1999). We video-recorded the swimming behaviour for 1 h upon introduction, while the actual exploration assessment was done for 5 min, starting from the moment in which the fish stopped freezing and showed normal swimming behaviour. Recording each individual for 1 h after introduction into the test tank ensured enough video material even if an individual would have shown a relatively long freezing time in the beginning. However, for our test individuals, freezing times were rather short and no individual had to be excluded because of extensive freezing (median \(\pm\) IQR single treatment: 18 \(\pm\) 66 s; group treatment: 21.5 \(\pm\) 85 s). In the single treatment, one individual showed the maximum freezing time of 17 min, which was still well within the range of our filming period. For video recordings, we used a GoPro Hero7 Black camera, which was mounted 1 m above the test tank. No observer was present in the room during the video recordings.

2.4. Video analysis

To translate the recorded swimming pattern into \(x\)-\(y\) coordinates, we used the tracking program *FishyGrid* developed by one of the authors (C.G.). The total tank area (bottom) was described as a grid with \(x = 21\) and \(y = 7\), which was chosen according to the size of the fish and the tank dimensions. The program follows the fish and saves all \(x\)-\(y\) coordinates (squares) that were covered by the fish within the given time. Output files were then processed by the python script *curiousfish* (developed by C.S.-T.; all scripts are available on GitHub: github.com/CariNCody/TrackAndVisualizeBehavior). The variable area explored (\(=\) exploration) was calculated as: Total sites/\((x \times y)\), which can theoretically range from 0 to 1 (the word ‘site’ refers to an individual grid cell). High values of ‘area explored’ indicate a high exploration tendency. Practically, the exact value of 0 does not exist in this study because no
fish stayed in freezing behaviour without moving at all for the entire test time. Otherwise, these fish would have been excluded from the statistical analyses. This basic criterion is crucial in order to disentangle exploration behaviour from acute stress responses, which are closer related to the shyness-boldness axis than to the explorative-non explorative axis, which was the focus of this study.

The second variable we calculated was thigmotaxis, defined as: \((q - p)/(q + p)\), with \(q = \text{Border sites}/(42 + 10)\), and \(p = (\text{Total sites} - \text{Border sites})/((x \times y) - 42 - 10)\). The sum of 42 and 10 describes the total number of grid cells along the walls of the test tank, which refer to the border sites (see definition of the \(x\)-\(y\) coordinates, describing the grid, above). A value of 1 for thigmotaxis indicates pure wall hugging, i.e., the test fish swims along the wall for the entire testing time, whereas a value of \(-1\) describes a situation in which the fish exclusively explored the central area of the tank. A value of 0 indicates no preference between border and central sites. The formula accounts for the bias that border sites are less abundant than central sites in our test tank.

### 2.5. Statistical analyses

The focus of this study was to investigate whether exploratory behaviour of a novel environment increases in the presence of a conspecific (positive social facilitation). To test whether exploratory behaviour differed between the two treatments ‘single’ and ‘group’, we applied a Wilcoxon matched pairs test on the ground that our response variable showed a deviation from normal distribution (Shapiro–Wilk test: \(W = 0.939; p = 0.032\)). Since our sample size was rather small and the deviation from normal distribution was not very strong, we performed the more powerful \(t\)-test as a backup. The results were not qualitatively different from those of the non-parametric test.

Another interesting variable that is associated with anxiety in novel situations is thigmotaxis (= wall hugging). It is assumed that if individuals swim very close to the walls for most of the time, their swimming pattern is strongly driven by anxiety. To test whether thigmotaxis is different between our treatments, we conducted a paired \(t\)-test (Shapiro–Wilk test: \(W = 0.953; p = 0.095\)).

All test fish were given one week in between the two behavioural tests, which we applied to minimize habituation effects (Montgomery, 1953). A habituation effect could either increase exploration (due to less fear) or
decrease it (due to less excitement) during the second behavioural assessment because of habituation to the test design. This well-known effect can potentially lead to misinterpretation of the results, which might then be rather driven by habituation than by the actual treatment. In order to account for this possibility, we randomized the order of treatments (half of the fish first did the single and then the group treatment and vice versa for the other half) and, additionally, conducted a Wilcoxon matched pairs test in which we did not compare single versus group treatment, but first to second behavioural test.

Finally, we tested if the change in exploratory tendency from single to group treatment (social facilitation) is dependent on the single exploratory tendency. This was done to address the question of whether novelty-averse (= low exploration) individuals were more affected by social facilitation than novelty-seeking (= high exploration) ones. To this end, we ran a Pearson correlation between ‘exploration single treatment’ and the difference in exploration between both treatments as: exploration(single)–exploration(group).

All statistical tests were performed in the R environment v4.0.2 (R Core Team, 2021). Figures were designed with Prism9 Graph Pad and Python v3.7.4 using the packages Pandas, Numpy, and Altair.

2.6. Ethical note

The experiments comply with the current laws on animal experimentation. All experimental treatments were performed under permit No. 2356 issued by the cantonal veterinary office Basel-Stadt.

3. Results

When comparing exploratory behaviour between the single and the group treatment we found that, contrary to our expectations, there was no difference in exploratory tendency between the treatments (Wilcoxon matched pairs test: $V = 136.5$, $p = 0.247$, $N = 20$, Figure 2a). The same was observed for thigmotaxis (paired t-test: $t = 0.859$, $p = 0.401$, $N = 20$, df = 19, Figure 2b).

While Figure 2 shows that the social context, in which the test fish explored its environment, did not affect average exploratory tendency, a visualization on the individual level provides a more detailed view on the results (Figure 3). It appears that the direction and extent of the change in behaviour across both behavioural tests was highly individual. While some individuals showed a rather consistent exploratory behaviour across both tests (slope...
Figure 2. The same ($N = 20$) female individuals were tested for their exploratory behaviour in a single and in a group treatment. (a) Exploratory behaviour was lower in the group treatment (median ± IQR: $0.53 ± 0.29$) than in the single treatment ($0.64 ± 0.31$), however, the difference between treatments was not statistically significant (Wilcoxon matched pairs test: $V = 136.5; p = 0.247$). (b) Thigmotaxis was reduced in the group ($−0.08 ± 0.38$) versus the single treatment ($0.17 ± 0.64$) but also not statistically significant (paired $t$-test: $t = 0.859, p = 0.401$). Boxplots show min–max with medians values.

close to 0), most test fish either increased or decreased considerably in exploration when being with a conspecific (steep positive or negative slope). These individuals strongly synchronized their swimming behaviour with the conspecific (i.e., remained closely together and, thus, explored very similar parts of the test tank) as visualized in Figure 4. Despite a synchronized swimming pattern, no further interactions such as aggressive behaviour were observed (see video at 10.6084/m9.figshare.19115717).

Notably, the strength and direction of behavioural change across treatments was strongly dependent on the exploratory tendency that individuals showed in the single treatment ($r_p = 0.862, p < 0.001, N = 20$). Individuals with a low exploration tendency in the single treatment were more prone to be affected by positive social facilitation than highly explorative ones (Figure A1 in the Appendix).

Having compared exploratory behaviour between first and second behavioural test, there is no reason to assume that habituation had affected our results (Wilcoxon matched-pairs test: $V = 126, p = 0.44, N = 20$).
Figure 3. Reaction norm plots showing the behavioural change between single and group treatment as individual lines. The steeper the slope of the line, the bigger the difference in behaviour across the two tests for the respective individual. The colour of a line represents the same individual in both plots. (a) Exploratory behaviour; 7 fish increased their exploratory behaviour in the presence of a conspecific, while 13 explored a larger ratio of the test tank when being alone. (b) Thigmotaxis (= wall-hugging); 14 test fish showed less wall-attached swimming behaviour in the group treatment, while 6 individuals had increased thigmotaxis when being with a conspecific.

4. Discussion

Exploration of the given environment in order to acquire crucial resources such as food, shelter or mating partners belongs to the deeply conserved behavioural axes ubiquitous in the animal kingdom (Réale et al., 2007). Anxiety towards novelty impedes efficient exploration, which can result in fitness consequences for the individual itself and its offspring (Boon et al., 2007; Smith & Blumstein, 2008). In many social animal species, being close to conspecifics reduces anxiety (Faustino et al., 2017) and should, therefore, enhance an individual’s willingness to explore its environment (Moretti et al., 2015).

In this study, we quantified the exploratory tendency of 20 females of the highly social Green chromide cichlid (*E. suratensis*) and compared the degree of exploration of a novel environment between the situation of being alone (single treatment) and the situation in which the focal individual was
tested together with a conspecific female (group treatment). We predicted a higher exploration tendency in the group treatment based on the assumed reduction of fear. Moreover, we expected thigmotaxis, which is an indicator of fearful behaviour (Schnörr et al., 2012), to be elevated in the single treatment. Contrary to our expectations, the two groups neither showed a difference in exploratory behaviour nor in thigmotaxis.

Could these results be explained by the fact that our test individuals were identical or at least very similar with respect to influential traits like sex (all females), age (same clutch), size (age-dependent), genetics (same parents), and experience (all fish stemmed from the same stock tank)? Previous studies on socially-dependent decision-making found that behavioural changes due to the presence of conspecifics are of variable strength and dependencies. For instance, male Atlantic mollies (Poecilia mexicana) change their behaviour related to their mate preference in the presence of competitors (= audience effect), while females do not (Plath et al., 2009). These behavioural changes
in males were shown to be dependent on the sexual activity of the competitor (Bierbach et al., 2011) and the risk-taking behaviour of the focal fish itself (Bierbach et al., 2015). With the choice of our test animals, we intended to reduce confounding factors like competition, sexual interaction and hierarchy to a minimum. Did this decision result in a homogenization to a degree that individuals would not be different enough to influence each other? If this was the case, we would expect no or very little difference in exploratory behaviour across treatments. An alternative explanation is that social facilitation on exploratory behaviour increases with group size as shown in the mosquitofish (*Poecilia mexicana*; Ward, 2012). It might be that our group size of only two individuals was too small to reduce fear in focal individuals. However, also in this case, we would expect that the influence of conspecifics on the focal individuals’ exploration behaviour was small.

Interestingly, however, a closer inspection of the results at the individual level as well as of the original video material revealed that test fish influenced each other considerably, which is clearly visible by a highly synchronized swimming behaviour (see video at 10.6084/m9.figshare.19115717) and differences in exploratory tendency across treatments in most individuals (Figures 3 and 4). Contrary to our expectations, over half of the focal individuals exhibited a higher exploratory tendency in the single than in the group treatment, while the rest of the test individuals showed the opposite. Since the opposing behavioural changes cancel each other out, we do not see a treatment effect overall. Our results thus suggest that social facilitation can increase or decrease exploratory tendency in juvenile *E. suratensis*. An alternative explanation could be that conspecifics would induce positive social facilitation in all test fish but that focal individuals are pulled by two conflicting pressures in the group treatment: their tendency to explore the given environment and their need to stay close to the conspecific, the latter potentially limiting the focal individual to freely move around. This ‘restriction’ is probably only present until the group size becomes large enough that collective decision-making guides their movement.

Independent of which of these two mechanisms may explain the lack of an overall treatment effect in our study, the direction of the slope across treatments (due to simplicity referred to as positive or negative social facilitation) seems to depend on the behavioural type of the focal individual (see Uziel, 2007 for similar findings in humans). A more thorough discussion of
this relationship including the potential influence of the mathematical phenomenon ‘regression to the mean’ is provided in the Appendix. It remains speculative which other factors could play a role in shaping an individual’s propensity to exhibit more or less exploration in the presence of a conspecific. We tentatively argue that also the combination of behavioural types in the group treatment together with the degree of experience with the test design could contribute to the effect of social facilitation. Unfortunately, we were restricted in the number of available female fish and our sample size of 20 individuals does not allow the application of complex models due to the risk of overfitting (Babyak, 2004). It would be desirable to conduct further studies with a larger sample size and with emphasis on different predictors to better understand the complex phenomenon of social facilitation in non-human animals.

Our study emphasizes that it is important to carefully look at behavioural data on the individual level, since we are prone to overlook crucial parts of the often complex picture if analyses focus exclusively on the group/population level.

Acknowledgements

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References


Our study shows that the exploratory behaviour of female *Etroplus suratensis* are affected by the presence of a conspecific in an individual way. Both strength and direction of this effect varied among our test fish. One possible explanation could be that an individual’s behavioural type (here: exploratory tendency) influences how and to which degree the individual responds to social facilitation.

We correlated the exploratory tendency that our test fish showed in the single treatment with the change in exploratory tendency across treatments \((\text{exploration(single)} - \text{exploration(group)})\), which is depicted in the following Figure A1.

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**Appendix**


Figure A1. Association (Pearson’s correlation) between an individual’s exploratory behaviour in the single treatment and its behavioural change across both treatments. A value of zero means that an individual showed the exact same exploratory tendency across both treatments.

More extreme personality types from both ends of the spectrum (very non-explorative and highly explorative) adjusted their exploratory behaviour stronger across treatments than intermediate personality types. This observation is in line with previous studies in fish in which the tendency to adjust swimming pattern varied across personality types as well as across the combination of personality types between individuals (Harcourt et al., 2009; Nakayama et al., 2016; Bevan et al., 2018). A striking feature of swarm fish is their homogenization in swimming patterns yielding several fitness advantages, with the most important one being protection against predators (Pitcher, 1986). Since an individual’s exploration tendency, by definition, influences the movement pattern in novel situations, it can be assumed that this behavioural tendency should become more homogenized between individuals when being in a group, in order to be able to synchronize with each other. The results of our study indicate that more extreme behavioural types contributed more to this homogenization when being in a group than intermediate behavioural types.

However, the strength of this correlation should be interpreted with caution since the two variables are not independent of each other. The smaller the exploratory tendency in the single treatment, the more likely the behavioural change across treatments will be negative. Furthermore, we cannot exclude that the mathematical phenomenon called ‘regression to the mean’ (Barnett et al., 2005) contributed to the strong correlation visualised in Figure A1.