Social investigation and social novelty in zebrafish: Roles of salience and novelty

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Abstract

Social preference tests can be used to analyze variables that influence and modify social behaviors, and to investigate effects of substances such as medications, drugs, and hormones. They may become important tools for finding a valid model to study neuropsychiatric changes and to study human neurodevelopmental processes that have been impaired by social events. While a preference for conspecifics has been shown for different species, social novelty has been used as a model for anxiety-like behavior in rodents. The goal of this research was to understand the roles of stimulus salience (numerosity) and novelty in social investigation and social novelty tests in zebrafish (*Danio rerio* Hamilton 1822). We used a sequential design, in which animals are exposed first to a social investigation test (with dichotomous presentation of novel conspecifics vs. empty tank) and then to a social novelty test (with dichotomous presentation of the already known conspecific and a novel conspecific). In experiment 1, animals were presented to either 1 or 3 conspecifics as stimuli. In experiment 2, animals were observed in the social investigation and social novelty tests for 3 consecutive days. The results showed equivalence between 1 or 3 conspecifics in the social investigation test, and stronger preferences for a novel conspecific when only one animal is shown on each side. These preferences do not change with repeated test exposure, suggesting novelty to be a minor contributor to social investigation and social novelty in zebrafish.

Keywords: Social preference; Social investigation; Social novelty; Zebrafish

1 Introduction

In social animals, including zebrafish, social preference is a very common behavior, where the animal makes choices over their conspecifics (Liu et al., 2016). Social preference tests can be used to analyze variables that influence and modify social behaviors in vertebrates, and to investigate effects of substances such as drugs, toxicants, and hormones (Engeszer et al., 2004; Ogi et al., 2021). Furthermore, social preference tests can also be important tools to create valid models to study neuropsychiatric disorders and neurodevelopmental processes that impact social behavior (Maruska et al., 2019; Ogi et al., 2021; Soares et al., 2018).

The neuromolecular mechanisms underpinning social behavior are only likely to be fully understood using information obtained from various social, environmental, and physiological conditions (Oliveira, 2013; M. Taborsky et al., 2015). The first step in determining the structure of social behavior, thus, is to analyze preference and choices at the behavioral level.

Known as one of the most important model species in biology, the zebrafish is a small teleost belonging to the Cyprinidae family (Parichy, 2015) originating from tropical areas, mainly in India, and is becoming a strong name in areas of human diseases and in drug discovery and evaluation (Dodd et al., 2000; Gerlai, 2020; Stewart et al., 2014). Currently, the zebrafish is given as a large organism highly suitable for large-scale genetic manipulation, taking its importance to a higher level; furthermore, their embryos and larvae are even used for automated screening of high-yield drugs (Rinkwitz et al., 2011). With its numerous advantages regarding its use in laboratory research, the zebrafish is also an excellent tool for studies in the field of developmental biology thanks to its external development that allows visual analysis of the initiation performance of embryogenesis, enabling research with better results (Dooley & Zon, 2000; Roper & Tanguay, 2018).

The social behavior of zebrafish has been initially described from the point of view of shoaling, but soon the full complexity of sociality in this species was noticed (Maruska et al., 2019; Oliveira, 2013). Zebrafish are capable of cooperating with conspecifics (Pimentel et al., 2021) and of social learning (Lindeyer & Reader, 2010). The sight of conspecifics can be used as a reinforcer for zebrafish during operant conditioning (Al-Imari & Gerlai, 2008), suggesting that sociality is itself rewarding. Nonetheless, zebrafish are not indiscriminately attracted to all conspecifics, exhibiting social preferences for individuals similar in phenotype, age and size (Barba-Escobedo & Gould, 2012; Engeszer et al., 2004, 2007, 2008;

Rosenthal & Ryan, 2005; Saverino & Gerlai, 2008). Social preferences in zebrafish are associated with many different neurotransmitter and neuromodulator systems, including dopamine (Scerbina et al., 2012), serotonin (Barba-Escobedo & Gould, 2012; Ponzoni et al., 2016), isotocin (Nunes et al., 2020; Ribeiro et al., 2020), and endocannabinoids (Barba-Escobedo & Gould, 2012).

Social preferences in zebrafish are visually mediated, with body morphology and body movement representing important dimensions that modulate preferences (Engeszer et al., 2008; Nunes et al., 2020). One of these dimensions is the salience of social stimuli. In a social preference test in which animals were presented with animated images of conspecifics, presentation of a single conspecific led to swimming away from the screen, while presenting up to 8 conspecifics led to a strong preference for swimming near the screen (Fernandes et al., 2015); however, no differences were observed between different stimulus numerosities (3, 5, or 7 conspecific images). Preferences for live conspecifics have been tested with different numerosities as well, with a preference for larger stimulus shoals (Potrich et al., 2015; Seguin & Gerlai, 2017). Baseline activity levels (body motion) seem to influence this response, as testing preference for conspecifics that are in warm water (high body motion) induce a marked preference for larger shoals, while testing preference for conspecifics that are in cold water (low body motion) abolishes this preference (Pritchard et al., 2001).

In the cases in which higher numerosity of social stimuli has been associated with social preferences, stimulus fish were also socially novel (Potrich et al., 2015; Pritchard et al., 2001; Seguin & Gerlai, 2017). However, Barba-Escobedo and Gould (2012) also showed that zebrafish display preference for social novelty, using a sequential design: in the first stage of the test ("social interaction", SI), the choice presented is between a unfamiliar conspecific (obtained from a tank other than that of the focal fish) and an empty box, while in the second stage ("social novelty", SN) the original conspecific ('old stranger') is presented in opposition to another unfamiliar conspecific ('new stranger'); therefore, in the second stage the novelty of the 'old stranger' has already decreased, and the choice is between two conspecifics differing in novelty. Phenotype-dependent preferences for unfamiliar conspecifics were established, with AB and golden zebrafish showing preference both in the first stage (SI) and the second stage (SN)(Barba-Escobedo & Gould, 2012; Norton et al., 2019). The differences between social interaction and social novelty are relevant, given that zebrafish lacking a functional isotocin (oxytocin-like) receptor show normal preference in the SI test, but reduced preference in the SN test (Ribeiro et al., 2020). Moreover, behavior in the SN test is more correlated with novel object exploration than with social tendencies or

behavior in the SI test across six zebrafish strains (Gonçalves et al., 2022). Gonçalves et al. (2022) suggested that preferences during the SN test are part of a “general exploration module”, and analysis of single-nucleotide polymorphisms (SNPs) across the zebrafish strains reveal associations with this module and genes involved in neurotransmission (e.g., serotonin and dopamine receptors), neuromodulation (e.g., NPY, oxytocin), synaptic plasticity (e.g., neuroligins, neurexins) and epigenetic marking (e.g., methyl CpG binding protein 2). Social tendencies, indexed by preferences in the SI test, on the other hand, are associated with a more restricted set of SNPs, including polymorphisms in genes encoding serotonin receptors (*htr1aa* and *htr3a*), as well as neuroligins and neurexins (Gonçalves et al., 2022).

The neurobehavioral differences in terms of social preference and novelty preference, as well as variations in responsiveness to social stimuli with differing salience (numerosity) point to the need to better understand the architecture of zebrafish social preferences in the SI and SN tests. The aim of the present study was to understand whether numerosity affects preference in both SI and SN tests, and whether decreasing novelty by repeated exposure to the conspecifics is sufficient to decrease preference in both tests.

2 Materials and methods

2.1 Animals and housing

50 adult zebrafish from the longfin phenotype were used in the experiments. Outbred populations were used due to their increased genetic variability, decreasing the effects of random genetic drift that could lead to the development of uniquely heritable traits (Parra et al., 2009; Speedie & Gerlai, 2008). The populations used in the present experiments are expected to better represent the natural populations in the wild. Animals were bought from a commercial vendor (Fernando Peixes, Belém/PA) and arrived in the laboratory with an approximate age of 3 months (standard length = 13.2 ± 1.4 mm), being quarantined for two weeks, before the experiment begin, when animals had an approximate age of 4 months (standard length = 23.0 ± 3.2 mm). Animals were kept in mixed-sex tanks during acclimation, with an approximate ratio of 1:1 males to females (confirmed by body morphology). The breeder was licensed for aquaculture under Ibama's (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) Resolution 95/1993. Animals were group-housed in 40 L tanks, with a maximum density of 25 fish per tank, for the aforementioned quarantine before experiments began. Tanks were filled with non-chlorinated water at room temperature (28 °C) and a pH of 7.0-8.0. Lighting was provided by fluorescent lamps in a cycle of 14 hours of

light-10 hours of darkness, according to standards of care for zebrafish (Lawrence, 2007). Other water quality parameters were as follows: hardness 100-150 mg/L $CaCO_3$; dissolved oxygen 7.5-8.0 mg/L; ammonia and nitrite < 0.001 ppm. Potential suffering of animals was minimized by controlling for the aforementioned environmental variables and scoring humane endpoints (clinical signs, behavioral changes, bacteriological status), following Brazilian legislation (Conselho Nacional de Controle de Experimentação Animal - CONCEA, 2017). Animals were used for only one experiment and in a single behavioral test, to reduce interference from apparatus exposure. Experiments were approved by UEPA's IACUC under protocol 06/18.

2.2 Experiment 1: Numerosity of social stimuli in preference and novelty experiments

32 animals were used in Experiment 1, with 24 being used as focal fish, and 8 being used as stimuli in the different stages and experimental conditions. To assess whether zebrafish are sensitive to the number of unfamiliar conspecifics in both SI and SN tests, fish were exposed to either one or three unfamiliar conspecifics in each stage. Focal animals were transferred to glass tanks (13 x 13 x 17 cm) juxtaposed to two other glass tanks of equal dimensions, and visual access to these tanks was blocked by white plastic divisions. Animals were allowed to freely explore the tank for 5 min., after which the divisions were removed, allowing visual access to the side tanks. For half of the animals, one of the side tanks contained one unfamiliar conspecific, taken from a home tank different than that from which the focal animal was obtained. For the other half, one of the side tanks contained three unfamiliar conspecifics, again taken from a home tank different than that from which the focal animal was obtained. Animals were allowed to freely explore the tank for another 10 minutes; this represents the *social interaction* (SI) stage. Immediately after that time period, the plastic divisions were inserted between tanks again, and the empty tank was quickly substituted by a tank containing the same amount of fish as the occupied one. Plastic divisions were then removed again, and the fish was allowed 10 more minutes to explore the tank; this represents the *social novelty* (SN) stage. In both the SI and SN stages, the side in which the "stimulus fish" was presented was randomly chosen between trials, and counter-balanced. Randomization was performed using the online tool at http://jerrydallal.com/random/assign.htm. Behavior was recorded with a video camera (Sony® DCR-DVD650 DVD Camcorder) mounted by the side of the tanks. Video files were

then analyzed using automated tracking with TheRealFishTracker v. 0.4.0 (https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/), running in Windows 10. While experimenters were not blinded to group, automated video tracking helps to reduce bias. The following variables were extracted for both SI and SN stages:

1. **Total time near stimulus fish/shoal** (SI) **or "new stranger"** (SN) (s), defined as the third of the focal tank nearest to the tank occupied by conspecifics. This is considered the primary endpoint of both tests.
2. **Total time near empty tank (**SI) **or "old stranger"** (SN) (s), defined as the third of the focal tank nearest to the unoccupied tank in the first stage, or the tank occupied by the previous unfamiliar fish in the second stage.
3. **Erratic swimming** (º), defined as the absolute turn angle averaged throughout the stage. This endpoint reflects an anxiety-like or fear-like component (Kalueff et al., 2013).
4. **Swimming speed** (cm/s), averaged throughout the stage.

2.3 Statistical analysis for Experiment 1

Since we were interested in understanding whether a larger number of individuals produced stronger preferences, or whether novelty still remained the strongest driver of preference, equivalence tests (Lakens, 2017) were used instead of conventional t-tests in the case of the preference endpoints (Total time near stimulus fish/shoal (SI) or "new stranger" (SN); Total time near empty tank (SI) or "old stranger" (SN)). For the SI test, equivalence bounds were set as $d = |3.9|$, based on the standardized effect size (Cohen's d) calculated from the data on social preferences (SI) of AB zebrafish from Barba-Escobedo and Gould (2012). For the SN test, equivalence bounds were set as $d = |1.83|$, based on the standardized effect size (Cohen's d) calculated from the data on preference for social novelty (SN) of AB zebrafish from the same study. P-values smaller than 0.05 were considered indicative of equivalence and thus no stronger preference for any stimulus numerosity.

We were also interested in whether three individuals induced less erratic swimming, a measure of fear-like or anxiety-like behavior in zebrafish (Kalueff et al., 2013). Conventional one-tailed t-tests were used in that case, with p-values smaller than 0.05 considered indicative of decreased anxiety in the group exposed to the three individuals

instead of a single individual. Finally, potential locomotor effects were assessed by looking at group differences in swimming speed, with two-tailed t-tests used (not assuming a direction of the effect). In both cases, effect sizes were calculated as Cohen's *d* (Cohen, 1988).

2.4 Experiment 2: Habituation of novelty

18 animals were used in Experiment 2, with 12 animals used as focal fish, and 6 as stimuli in the different conditions (SI and SN) and days. To understand the role of novelty in the SI and SN tests, animals were exposed to the tests again for 3 consecutive days. This new exposure allowed us to evaluate the repeatability of the test. Animals were repeatedly exposed to the same procedure in Experiment 1, with a single conspecific as stimulus in both the SI and SN tests. Animals were isolated in individual 2 L tanks between trials.

2.5 Statistical analysis for Experiment 2

To analyze the stability of preference and the role of novelty in SI and SN habituation, repeated measures analyses of variance (ANOVAs) were applied, with day as within-subjects variable. *P*-values smaller than 0.05 were considered indicative of habituation. Effect sizes were calculated as partial eta-squared (η^2p) (Richardson, 2011).

3 Results

3.1 Experiment 1

In the SI test (Figure 1), TOST equivalence tests were not able to discard the hypothesis of non-equivalence between groups (<u>time near stimulus</u>: TOST upper: $t_{[df = 18.2]} = -9.15$, $p < 0.001$; TOST lower: $t_{[df = 18.2]} = 9.95$, $p < 0.001$; <u>time far from stimulus:</u> TOST upper: $t_{[df = 20.95]} = -9.01$, $p < 0.001$; TOST lower: $t_{[df = 20.95]} = 10.10$, $p < 0.001$). Conventional *t*-tests were also unable to detect differences between groups (<u>time near stimulus</u>: $t_{[df = 18.2]} = 0.4$, $p = 0.695$; <u>time far from stimulus:</u> $t_{[df = 20.95]} = 0.55$, $p = 0.59$). Thus, there is no evidence that, in the current paradigm, zebrafish show stronger preferences for 3 conspecifics vs. an empty tank than 1 conspecific vs. an empty box in the SI test. One-tailed one-sample *t*-tests

were made to confirm preference for conspecifics; in the group exposed to a single conspecific in each side, a significant preference for the conspecific was found ($t_{[df = 11]} = 4.0$, $p < 0.001$, $d = 1.15$, 95%CI[0.4, 1.88]). Similar results were found for the group exposed to 3 conspecifics on each side ($t_{[df = 11]} = 5.79$, $p < 0.001$, $d = 1.67$, 95%CI[0.76, 2.55]) One-tailed *t*-tests did not detect differences between groups in erratic swimming ($t_{[df = 22]} = 0.7$, $p = 0.753$, $d = 0.28$, 95%CI[-0.53, 1.09]) or speed ($t_{[df = 22]} = -0.75$, $p = 0.769$, $d = -0.31$, 95%CI[-1.11, 0.51]).

Different results were found for the SN test (Figure 2). TOST equivalence tests suggest that the hypothesis of non-equivalence between groups is false for the time spent near the "new stranger", but not time spent near the "old stranger" (<u>time near stimulus</u>: TOST upper: $t_{[df = 21.46]} = -2.21$, $p = 0.019$; TOST lower: $t_{[df = 21.46]} = 6.76$, $p < 0.001$; <u>time far from stimulus:</u> TOST upper: $t_{[df = 21.03]} = -4.72$, $p < 0.001$; TOST lower: $t_{[df = 21.03]} = 4.24$, $p < 0.001$). Conventional *t*-tests were also able to detect differences between groups (<u>time near stimulus</u>: $t_{[df = 21.46]} = 2.28$, $p = 0.033$; <u>time far from stimulus:</u> $t_{[df = 21.03]} = -0.24$, $p = 0.814$). One-tailed one-sample *t*-tests were made to confirm preference for novelty; in the group exposed to a single conspecific in each side, a significant preference for the "new stranger" was found ($t_{[df = 11]} = 2.04$, $p = 0.033$, $d = 0.59$, 95%CI[-0.04, 1.19]). However, in the group exposed to 3 conspecifics on each side, no preference could be found ($t_{[df = 11]} = 0.03$, $p = 0.489$, $d = 0.01$, 95%CI[-0.56, 0.57]). Thus, there is evidence that, in the current paradigm, zebrafish show stronger preferences for a novel conspecific in the SN test only when one conspecific is shown on each side. One-tailed *t*-tests did not detect differences between groups in erratic swimming ($t_{[df = 22]} = 1.1$, $p = 0.282$, $d = 0.45$, 95%CI[-0.38, 1.26]) or swimming speed ($t_{[df = 22]} = -0.06$, $p = 0.953$, $d = -0.02$, 95%CI[-0.82, 0.78]).

3.2 Experiment 2

In the SI test (Figure 3), a repeated-measures ANOVA detected no significant effects of test day ($F_{[2, 22]} = 0.63$, $p = 0.541$, $\eta^2p = 0.05$) for the time spent near the conspecific. Likewise, no significant effects of test day were found for the time spent far from the conspecific ($F_{[2, 22]} = 0.55$, $p = 0.582$, $\eta^2p = 0.05$). No effects of test day were found for erratic swimming ($F_{[2, 22]} = 1.15$, $p = 0.335$, $\eta^2p = 0.09$) or swimming speed ($F_{[2, 22]} = 0.08$, $p = 0.928$, $\eta^2p = 0.01$).

In the SN test (Figure 4), no significant differences were detected between test days for time spent near the "new stranger" ($F_{[2, 22]} = 0.99$, $p = 0.389$, $\eta^2p = 0.08$). Differences were also absent for the time spent near the "old stranger" ($F_{[2, 22]} = 0.33$, $p = 0.725$, $\eta^2p = 0.03$). Finally, no effects were found for erratic swimming ($F_{[2, 22]} = 0.65$, $p = 0.532$, $\eta^2p = 0.06$) or swimming speed ($F_{[2, 22]} = 0.1$, $p = 0.902$, $\eta^2p = 0.01$).

4 Discussion

The present experiments assessed the role of numerosity and habituation in the zebrafish social interaction (SI) and social novelty (SN) tests. It was found that zebrafish show stronger preferences for 3 conspecifics vs. an empty tank than 1 conspecific vs. an empty box in the SI test, but stronger preferences for a novel conspecific in the SN test only when one conspecific is shown on each side. These preferences did not change with repeated exposure to the test apparatuses.

Preference for numerosity has been shown in other studies in fish, usually as evidence of the cognitive capacities of teleosts (Potrich et al., 2015; Seguin & Gerlai, 2017). In the present experiments, we were interested in whether preferences were stronger for individuals presented with a single conspecific or a shoal, as an index of social stimulus salience. Thus, we were interested in whether zebrafish would adjust their social preferences depending on the numerosity of the stimulus. In the SI test - in which the individual chooses between one or more novel conspecifics and an empty tank (Barba-Escobedo & Gould, 2012), animals consistently preferred the shoal. Social stimulus novelty is an important factor in this test, however, and the presence of a larger shoal could represent not a more reinforcing stimulus, but simply a more novel social context. In the SN test, individuals must choose between the conspecific which was previously shown and a novel conspecific; thus, the novelty of the previous interaction decreases, while individuals are shown another social stimulus with higher novelty. In that situation, the novelty of the first unknown conspecific ("stranger 1") decreases, while the novelty of the second unknown conspecific ("stranger 2") is at its highest.

We found that increasing stimulus salience led to higher preference during the SI test, but not during the SN test. Two interpretations are equally possible: that, in situations with higher numerosity, novelty discrimination is harder; or that social salience is a stronger

motivator for social preference than novelty in zebrafish. Novelty is also anxiogenic for most vertebrates (Maximino et al., 2012; Montgomery, 1955; Montgomery & Monkman, 1955); novel social situations are inherently anxiogenic, since they involve an approach-avoidance conflict in which potential benefits of social interactions with novel individuals are counterbalanced by potential risks (Soares et al., 2018). Socially competent individuals of a given species are more likely to be sensitive to the risk-benefit balance and assess the situation, plastically changing its behavior to better adapt the actual social context (B. Taborsky & Oliveira, 2012). Nonetheless, preference was found to be equivalent after repeated exposures to the apparatus (that is, preference in the SI test did not habituate) or to social novelty (that is, preference in the SN test also did not habituate). Moreover, no effects were observed of either salience/numerosity (Experiment 1) or habituation (Experiment 2) on typical measures of anxiety-like behavior, such as erratic swimming and freezing.

Taken together, these results suggest that social preference is guided by a complex combination of stimulus salience and novelty in zebrafish. These results underline the complexity of zebrafish social behavior and the need to understand the motivational basis of this process. Moreover, it also points to practical considerations on using social preference (interaction and novelty) tests in neurobehavioral research. For example, the use of three individuals instead of a single individual is recommended. Moreover, since preference does not seem to habituate, repeated exposure can be made using the same individuals, which is advantageous to assess the effects of repeated treatments (including those that are common in pharmacological research).

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Figure captions

Figure 1 – Lack of effect of salience/numerosity, indexed as number of conspecifics used as social stimuli, in the social investigation test. (A) Total time near stimulus fish/shoal. (B) Total time near empty tank (SI). (C) Erratic swimming. (D) Swimming speed. The effect sizes of differences between individuals exposed to 1 conspecific and 3 conspecifics vs. an empty tank in the social interaction test is shown in the Gardner-Altman estimation plots. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on a floating axis on the right as a bootstrap sampling distribution. Cohen's *d* is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

Figure 2 – Stimulus salience/numerosity affects preference for a novel stranger in the social novelty test. (A) Total time near stimulus fish/shoal. (B) Total time near empty tank (SI). (C) Erratic swimming. (D) Swimming speed. The effect sizes of differences between individuals exposed to 1 conspecific and 3 conspecifics vs. an empty tank in the social interaction test is shown in the Gardner-Altman estimation plots. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on a floating axis on the right as a bootstrap sampling distribution. Cohen's *d* is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

Figure 3 – Stability of social preference in the social investigation test after repeated exposure for three days. (A) Total time near stimulus fish/shoal. (B) Total time near empty tank (SI). (C) Erratic swimming. (D) Swimming speed. The effect sizes (Cohen's *d*) for 2 comparisons (days 2 and 3) against the shared control (day 1) are shown in the Cumming estimation plots. The raw data is plotted on the upper axes. On the lower axes, mean differences are plotted as bootstrap sampling distributions. Each mean difference is depicted as a dot. Each 95% confidence interval is indicated by the ends of the vertical error bars. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

Figure 4 – Stability of social preference in the social novelty test after repeated exposure for three days. (A) Total time near stimulus fish/shoal. (B) Total time near empty tank (SI). (C) Erratic swimming. (D) Swimming speed. The effect sizes (Cohen's *d*) for 2 comparisons (days 2 and 3) against the shared control (day 1) are shown in the Cumming estimation plots.

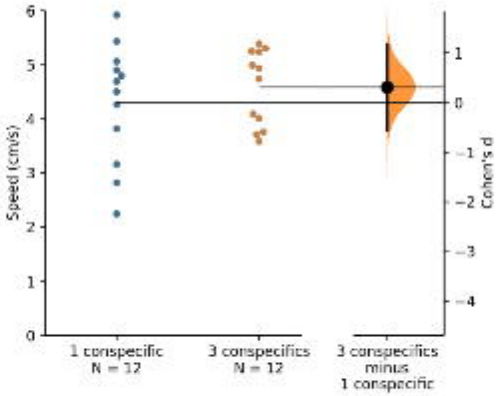
The raw data is plotted on the upper axes. On the lower axes, mean differences are plotted as bootstrap sampling distributions. Each mean difference is depicted as a dot. Each 95% confidence interval is indicated by the ends of the vertical error bars. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

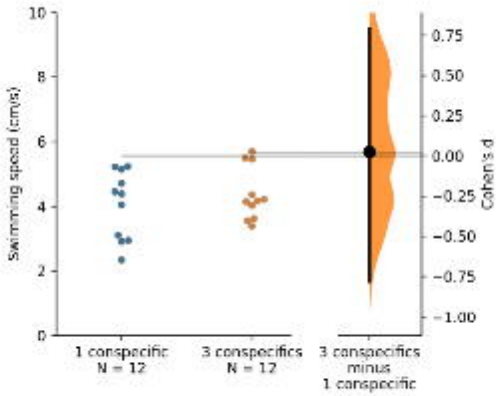
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific
Cohen's d

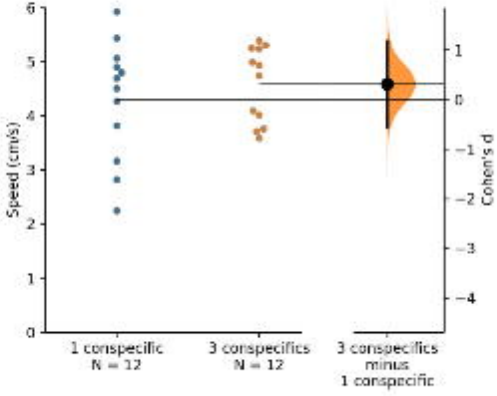
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific
Cohen's d

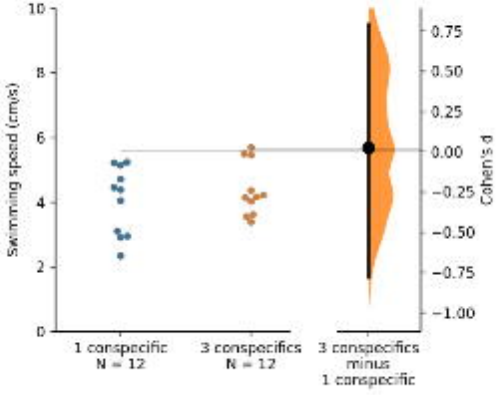
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific
Cohen's d

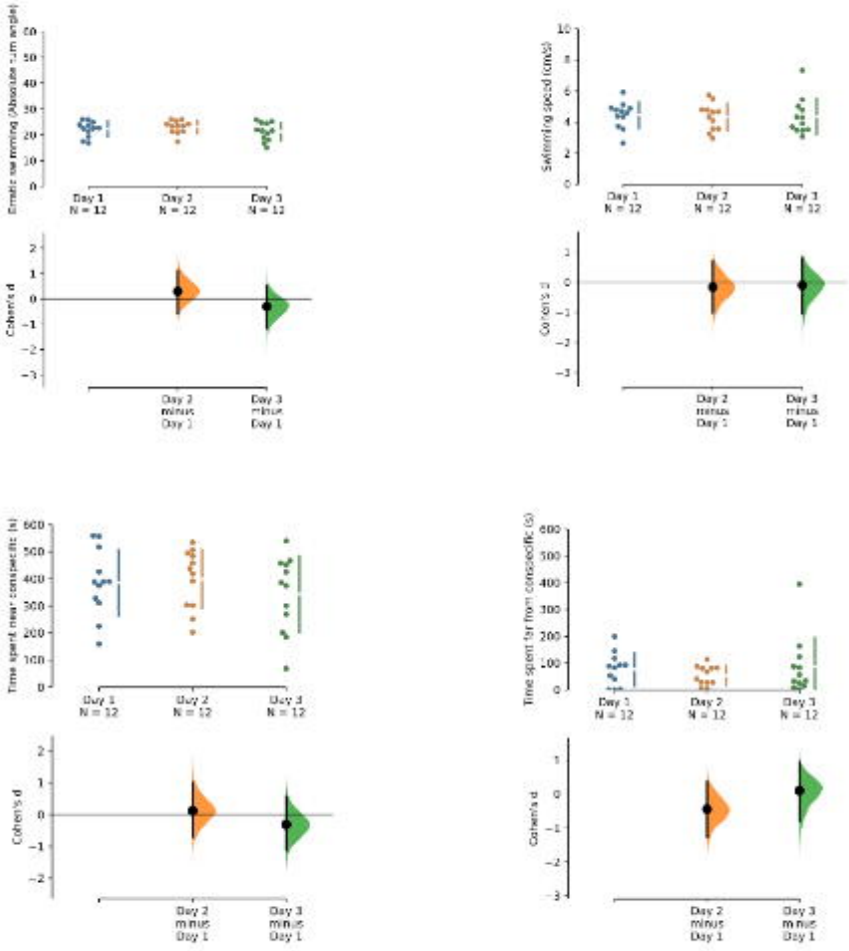
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific
Cohen's d

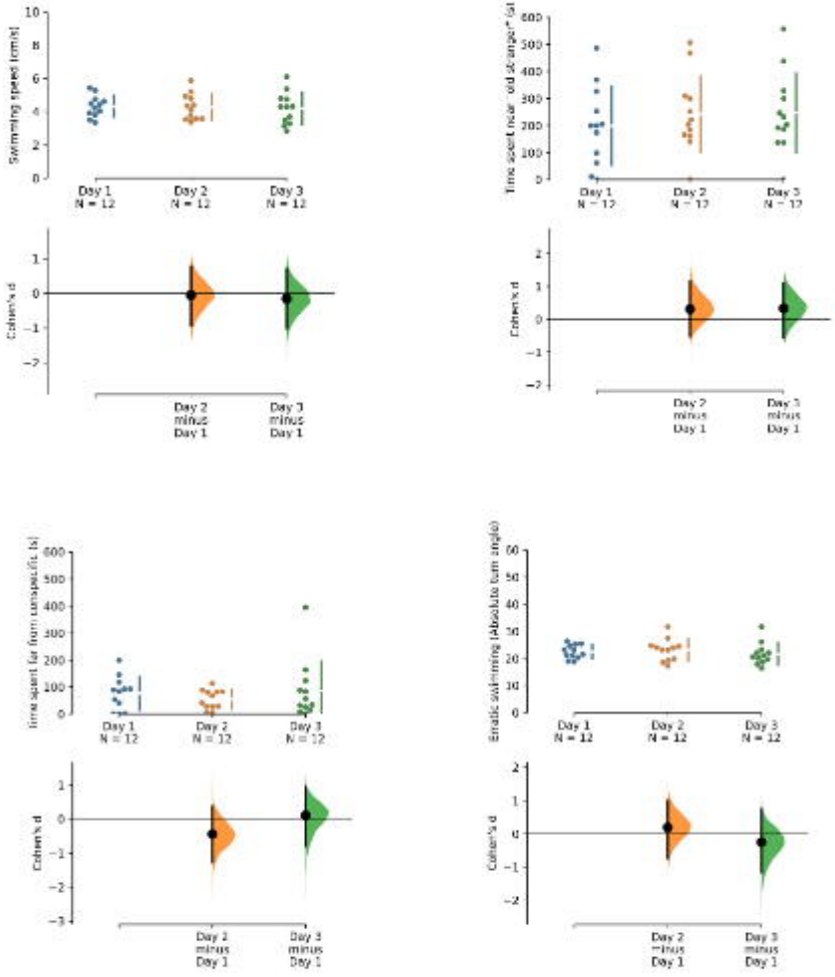

Speed (cm/s)
Cohen's d
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific


Swimming speed (cm/s)
Cohen's d
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific


Speed (cm/s)
Cohen's d
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific


Swimming speed (cm/s)
Cohen's d
1 conspecific
N = 12
3 conspecifics
N = 12
3 conspecifics
minus
1 conspecific


Erratic swimming (Absolute turn angle)
Day 1
N = 12
Day 2
N = 12
Day 3
N = 12
Cohen's d
Day 2
minus
Day 1
Day 3
minus
Day 1
Swimming speed (cm/s)
Time spent near conspecific (s)
Time spent far from conspecific (s)


Swimming speed (cm/s)
Day 1
N = 12
Day 2
N = 12
Day 3
N = 12
Cohen's d
Day 2
minus
Day 1
Day 3
minus
Day 1
Time spent near old stranger* (s)
Day 1
N = 12
Day 2
N = 12
Day 3
N = 12
Cohen's d
Day 2
minus
Day 1
Day 3
minus
Day 1
Time spent far from conspecific (s)
Day 1
N = 12
Day 2
N = 12
Day 3
N = 12
Cohen's d
Day 2
minus
Day 1
Day 3
minus
Day 1
Erratic swimming (Absolute turn angle)
Day 1
N = 12
Day 2
N = 12
Day 3
N = 12
Cohen's d
Day 2
minus
Day 1
Day 3
minus
Day 1