



Research article

Proto-arithmetic abilities in zebrafish (*Danio rerio*)

Davide Potrich^{a,*}, Lorenza Montel^a, Gionata Stancher^b, Greta Baratti^a,
Giorgio Vallortigara^a, Valeria Anna Sovrano^{a,c,**}

^a CIMEC, Center for Mind/Brain Sciences, University of Trento, 38068, Rovereto, (TN), Italy

^b Fondazione Museo Civico di Rovereto, 38068, Rovereto, (TN), Italy

^c Department of Psychology and Cognitive Science, University of Trento, 38068, Rovereto, (TN), Italy

ARTICLE INFO

Keywords:

Quantity
Numerousness
Number sense
Arithmetic
Zebrafish

ABSTRACT

The increasing use of zebrafish (*Danio rerio*) as a model for studying the neural bases of numerical/quantity abilities pushes toward the development of fast and reliable behavioral tasks for this species. Here, we investigated the spontaneous use of proto-arithmetic in quantity discrimination in zebrafish taking advantage of their shoaling behavior. Male fish underwent preference choice tests in which sets of live female conspecifics sequentially disappeared one by one behind one of two opaque identical panels. Fish spontaneously approached the panel occluding the larger set in a “1 vs. 2” comparison, but failed at “2 vs. 3” and “2 vs. 4”. Limited to an overall amount of three elements in the two groups, zebrafish appeared to be able to deal with additions and subtractions, also suggesting the implicit understanding of an “empty set” (zero) concept. The velocity and the sequential/simultaneous presentation of the stimuli affected the spontaneous preference towards the group with the largest quantity.

1. Introduction

The ability to estimate and discriminate the number of objects in a scene is widespread in the animal kingdom. Such a sense of number [1] is fundamental for humans and other animals to successfully face ecological needs such as food intake, sociality, and reproduction (for a review see Refs. [2–5]). The non-symbolic ability to estimate the number of elements in a set seems to be supported by an “Approximate Number System (ANS)”, which is based on a numerical ratio dependence, thus obeying Weber’s law: as the numerical ratio increases it gets more difficult to discriminate the numerosity of two sets [6,7]. Besides the ANS, evidence suggests that small numerosities (<4) might be precisely tracked and processed in parallel by the so-called “Object File System (OFS)” [8,9].

Simple numerical abilities involve recognizing and comparing quantities (e.g., determining which set is larger) without performing operations, mainly relying on the approximate number system (ANS). In contrast, proto-arithmetical abilities go further by incorporating basic arithmetic operations like addition and subtraction engaging intricate cognitive processes, as it requires dual-levels of information processing. The initial level involves the representation of numerical attributes, while the subsequent level entails the mental manipulation of these representations in working memory [10]. The ability to perform proto-arithmetic encompasses basic cognitive skills related to numerical processing, while proper arithmetical cognition involves understanding the discrete, infinite nature of natural numbers and progressing toward formal arithmetic, including theorem-proving about all natural numbers [11]. In

* Corresponding author.

** Corresponding author. CIMEC, Center for Mind/Brain Sciences, University of Trento, 38068 Rovereto (TN), Italy.

E-mail addresses: davide.potrich@unitn.it (D. Potrich), valeriaanna.sovrano@unitn.it (V.A. Sovrano).

infants, a pioneering study on the development of basic arithmetic skills was performed by Karen Wynn (1992) [12] with 5-month-old children, who observed the ability to make arithmetic operations of objects (i.e., puppets hiding behind an opaque screen) involving basic arithmetic operation (e.g., $1 + 1$; $2 + 1$; $2 - 1$). Similar results have been obtained when both visual and auditory stimuli (e.g., 1 object + 1 sound = 2 objects, or 1 object + 2 sounds = 3 objects) were used, suggesting that infants show some abstract reasoning abilities. The evidence gathered also shows that the absence of language does not impair the ability to perform basic numerical calculations (meta-analysis by Christodoulou and Moore (2017) [13]); this is further confirmed by evidence in non-human animals.

An ability to manipulate numerical quantities to solve arithmetic operations has been documented in non-human primates [14–16], birds [17,18], spiders [19], dogs [20], bees [10], and fish [21]. Non-humans usually are tested in a sequential presentation of attractive objects (e.g., food or social stimuli) with spontaneous choice motivated by reaching/rejoining the largest quantity. Such arithmetical abilities might be present already at birth: 3-day-old chicks (*Gallus gallus domesticus*) can choose the larger group of imprinting objects after seeing individual objects disappearing behind one of two panels, with two different sets forming and including addition and subtraction of elements [18].

Bees and some fish (cichlid fish species *Pseudotropheus zebra*, Boulenger, 1899; freshwater rays *Potamotrygon motoro*, Müller & Henle, 1841) can be trained to use color as context-dependent information for addition and subtraction tasks [10,21]. In these studies, animals were trained in two-alternative forced-choice in a delayed matching-to-sample procedure. Subjects were required to execute either an addition or subtraction operation, based on the presentation of visual stimuli depicting a group of elements in two distinct colors (i.e., blue or yellow), with the color signaling which specific arithmetic operation to be performed.

Despite the abovementioned evidence in cichlids and stingrays, the knowledge gathered so far regarding the ability to operate arithmetical operations in fish species is quite limited. In particular, no evidence has been collected so far for the model species that offers the best opportunity to study the neural and genetic origins of such abilities, namely the zebrafish (*Danio rerio*, Hamilton, 1822; review by Messina et al. (2022) [5]). Zebrafish offer important advantages: their brain architecture is largely homologous to that of mammals and their genome has been completely sequenced [22].

The present study aims to expand comparative research on numerical abilities in zebrafish by exploring the presence of elementary arithmetic using a spontaneous choice paradigm. This does not involve any form of learning and it is made possible in zebrafish by exploiting their natural gregarious behavior shoaling [23]. In their natural habitat, zebrafish exhibit shoaling behavior, forming social groups known as "shoals," which offer several adaptive benefits. Shoaling enhances foraging efficiency and predator avoidance by increasing vigilance within the group, allowing for more effective predator detection and providing a higher likelihood of escape during attacks, a phenomenon referred to as the dilution effect (see review in Ref. [24]). Shoaling behavior can be shaped by factors like vegetation, water flow, temperature, age, location, shoal size, predation, early life experiences, and food availability [25–29]. Regarding the development of shoaling behavior in zebrafish, observable shoaling responses typically appear around 14–15 days post-fertilization, while social preferences can start as early as 6–8 days [30,31]. Early experiences are crucial, as fish raised in isolation show reduced social preferences in adulthood [29,32]. Previous studies, such as those of Potrich and colleagues (2015) [33] and Seguin and Gerlai (2017) [34], have shown that zebrafish spontaneously approach the larger numerical of two simultaneous groups of conspecifics, provided that the numerical ratio between the two groups does not exceed 0.67 (i.e., 2 vs. 3, 4 vs. 6).

In our study, we tested zebrafish motivation to join the larger group of live female conspecifics (not directly visible at the moment of

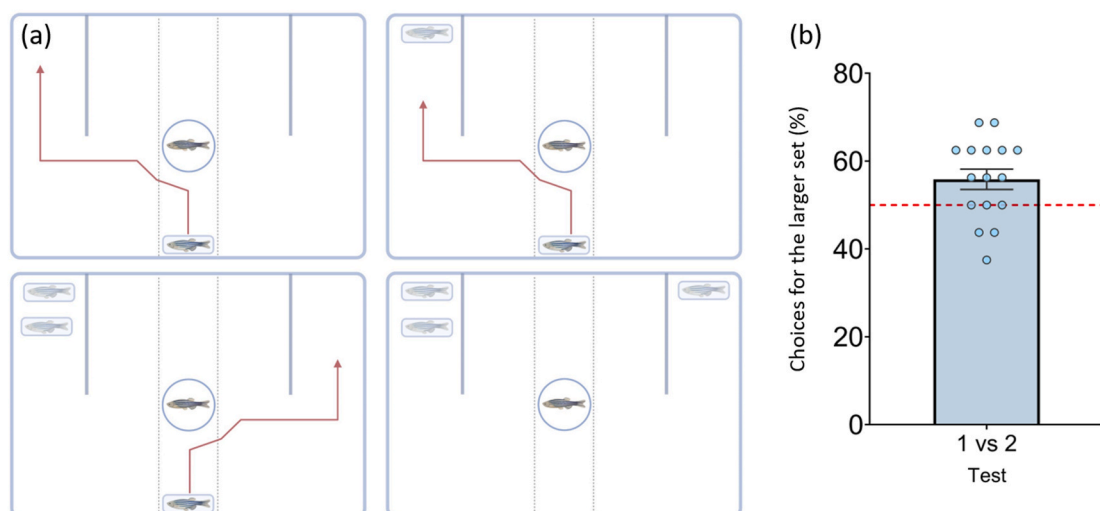


Fig. 1. (a) Schematic representation of the experiment in the 1 vs. 2 condition with fish hidden at the time of choice: a conspecific is hidden behind the left opaque panel; a second fish, moving along the same trajectory is placed behind the left panel; a third conspecific is placed behind the right panel; all fish are in the final position, before releasing the experimental fish. Left-right sides and groups' orders of presentation were counter-balanced across trials. (b) Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish.

the choice) after watching and keeping track of sequential presentations of single fish (each fish stimulus confined into a transparent box) hiding behind opaque panels and forming two different numerical groups. This task allows us to observe whether zebrafish can represent numerical quantities and apply mental operations such as addition and subtraction retaining their outcomes in working memory.

2. Results section

2.1. Experiment 1: Hidden conspecifics

The first experiment investigated whether zebrafish were capable of approaching the larger of two groups of conspecifics that were presented sequentially, one at a time, and hidden behind two opaque panels, making them no longer visible at the time of the experimental subject's choice. The test subject (a male individual) was confined within a transparent cylinder placed in the center of a rectangular tank, from where it could observe the sequential presentation of 3 social stimuli (individually placed inside small transparent boxes) one at a time as follows: two conspecifics (1 + 1) were hidden behind one panel, and one behind the opposite panel (see Fig. 1a). The speed of each individual's movement was kept constant. Then the approach of the test fish towards one of the two opaque panels was recorded (see Methods section for details).

2.1.1. Results and discussion

Choices were analyzed using a “generalized linear mixed model” (GLMM, see Methods section), employing a binomial distribution with the response towards the larger/smaller group as the dependent variable. The last movement (whether the last stimulus movement belonged to the larger or smaller group) was treated as a fixed effect, and subjects (FishID) as a random effect. The analyses of random effects did not show any influence on the statistical model (random intercept variance of the best fit: 1.14×10^{-12}), and no significant difference related to the order of stimulus movement (fixed effect) was found. This suggests adopting the simpler model, which takes into account only choices towards the larger/smaller group. The final estimate of the best model (logarithmic odds ratio) was 0.2355 ± 0.126 . The binomial test indicated a success probability of 55.9 %, $p = 0.07$ (95 % confidence intervals = 49.54–62.04), suggesting a non-significant difference compared to the control case (Fig. 1b).

The results of this initial experiment suggested that zebrafish failed to choose the larger group in the 1 vs. 2 comparison. Nevertheless, a tendency to approach the set composed of more females was apparent, albeit not significant. This outcome may be open to different explanations. First, animals might fail in the task of adding individual conspecifics (1 + 1 vs. 1) because monitoring and adding elements could be too challenging and demanding. A second possibility, however, could be related to the lack of motivation to

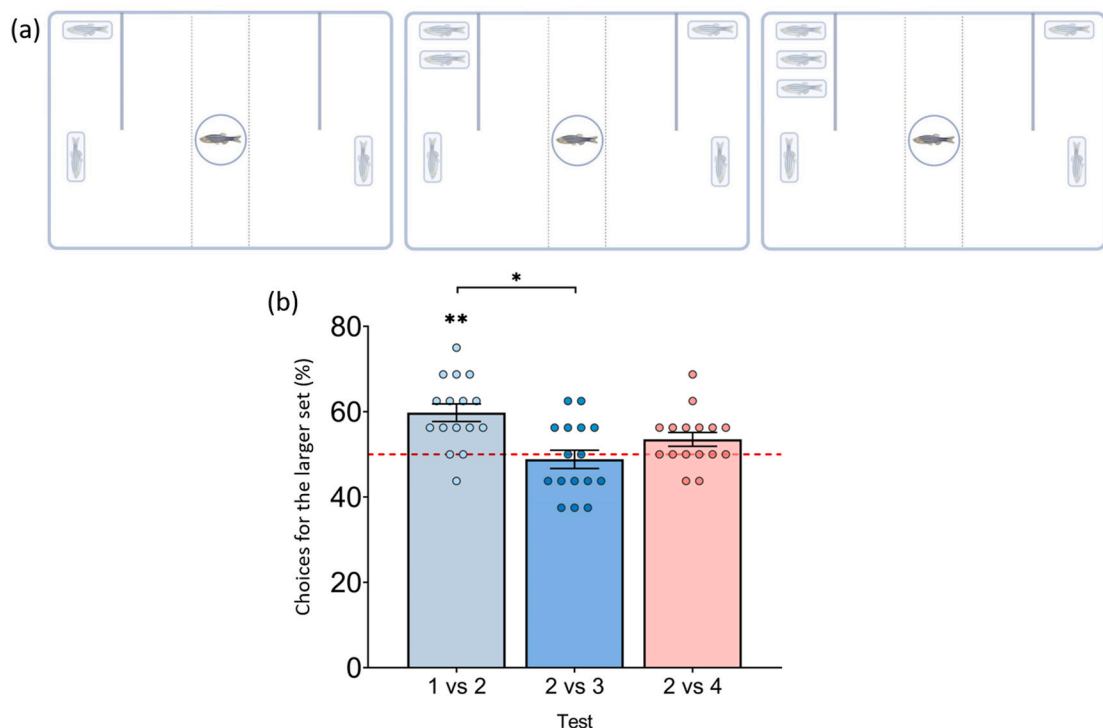


Fig. 2. (a) Schematic representation of the conspecifics' positioning in the tested condition with the last conspecifics visible (from left to right: 1 vs. 2, 2 vs. 3, and 2 vs. 4 comparisons). (b) Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish. Significant departures from chance level (50 %) are shown by asterisks (* $p < 0.05$, ** $p < 0.01$).

approach one of the two groups at the time of choice, as no fish were actually visible. All previous studies with fish involving numerical discrimination of groups of conspecifics were conducted with at least some conspecifics visible at the time of choice [33,35,36]. In the absence of visual stimulation at the time of choice, animals may show no motivation to reach one or the other of the two groups. Thus, in a second experiment, the procedure was modified by ending the last movement of each of the two groups near the occluding panel without hiding, thus leaving one stimulus visible to the experimental fish at the time of choice.

2.2. Experiment 2: Conspecifics partially visible

This experiment was conducted to understand whether, through a variation of the procedure used in Experiment 1, zebrafish showed an attraction towards the group with a greater number of conspecifics. The modification applied in this experiment consisted of hiding all the conspecifics behind the panels except the last one. In detail, the last stimulus for each group (either larger or smaller), once the movement trajectory was completed, was positioned near the opaque panel, without however any hiding, thus remaining fully visible to the experimental fish confined in the cylinder. In this way, at the moment of choice, the experimental subject could see one conspecific from both sides (Fig. 2a).

Three numerical comparisons were tested: “1 vs. 2” (1 vs. 1 + 1), “2 vs. 3” (1 + 1 vs. 1 + 1 + 1) and “2 vs. 4” (1 + 1 vs. 1 + 1 + 1 + 1).

2.2.1. Results and discussion

A generalized linear mixed model (GLMM) was applied using a binomial distribution with the response towards the larger/smaller group as a dependent variable, the test type (1 vs. 2, 2 vs. 3, and 2 vs. 4), and the last movement (performed by the major or minor group) as fixed effects, while the subjects (FishID) as a random effect. The statistical analysis highlighted a significant difference associated with the effect of the type of Test (χ^2 (2, $N = 48$) = 6.5, $p = 0.039$), but not with the last movement or its interaction with the type of Test. Post-hoc analyses highlighted a significant difference between the “1 vs. 2 – 2 vs. 3” conditions ($Z = 2.531$, $p = 0.0305$), but not between the “2 vs. 3 – 2 vs. 4” ($Z = -1.061$, $p = 0.5384$) and between “1 vs. 2 – 2 vs. 4” ($Z = 1.495$, $p = 0.2931$). The distributions of the animals’ choices were therefore considered and analyzed via binomial tests separately, depending on the discrimination performed (Fig. 2b). The binomial tests highlighted a significant difference compared to chance in the 1 vs. 2 condition (probability of success: 60.17 %, $p = 0.0019$, 95 % confidence intervals = 53.68–66.35), but not in the 2 vs. 3 condition (probability of success: 48.83 %, $p = 0.7547$, 95 % confidence intervals: 42.6–55.1) and 2 vs. 4 (probability of success: 53.52 %, $p = 0.288$, 95 % confidence intervals: 47.2–59.8).

When exposed to groups of conspecifics where the last stimulus remained visible to the test subject, zebrafish showed a preference for the larger numerical group in the “1 + 1 vs. 1” comparison, but not when exposed to the 2 vs. 3 (1 + 1 vs. 1 + 1 + 1) and 2 vs. 4 (1 + 1 vs. 1 + 1 + 1 + 1) conditions. The inability to choose the larger group in the 2 vs. 3 comparison compared to the success in

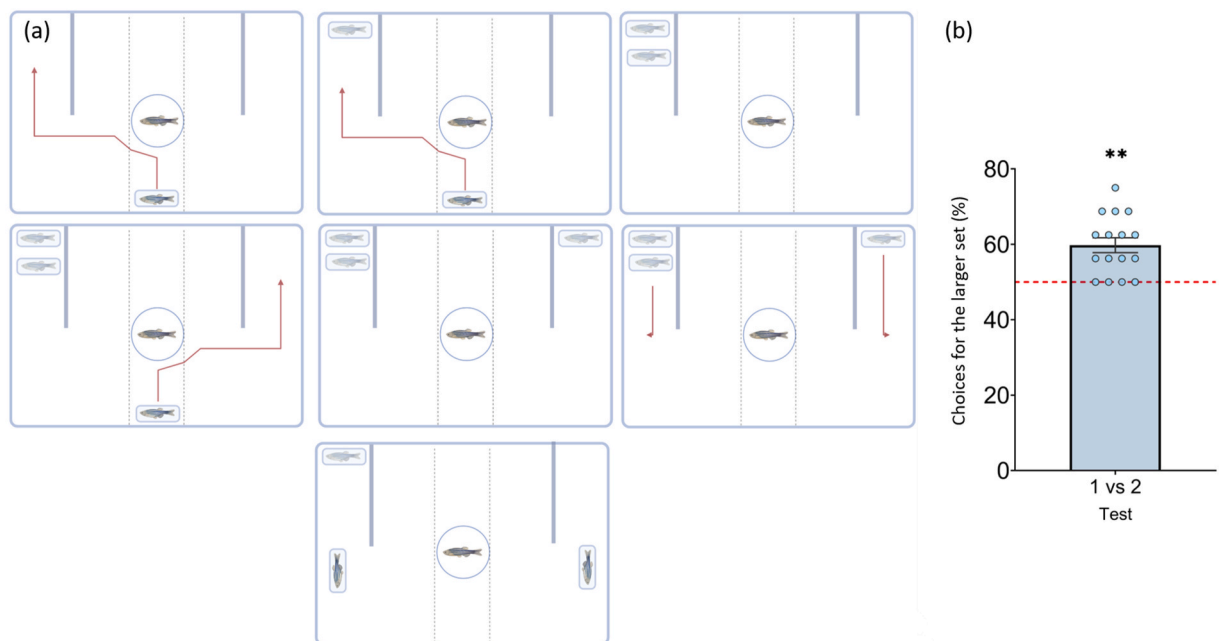


Fig. 3. (a) Schematic representation of conspecific positioning in condition 1 vs. 2 in Experiment 3a. After being positioned one at a time with constant speed, one fish on each side of the apparatus becomes visible again. This presentation includes both addition and subtraction of elements. (b) Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish. Significant departures from chance level (50 %) are shown by asterisks (** $p < 0.01$).

discriminating between 1 vs. 2 could be due to the unfavorable numerical ratio (respectively, 0.67 and 0.5). However, fish also fail in the comparison between 2 vs. 4 conspecifics, despite the numerical ratio being identical to the 1 vs. 2 condition (0.5). A possible explanation could concern the growing cognitive load, which requires paying attention to a large number of elements presented individually and for a total amount of time that is probably too long. In the 1 vs. 2 discrimination, in fact, the overall time elapsed between the entry into the apparatus of the first conspecific and the release of the experimental fish was equal to approximately 40 seconds, while it could extend up to 1 minute in the case of the 2 vs. 4 comparison. However, it should be noted that, although not significant, the trend in the 2 vs. 4 condition was in the direction of the larger group, which was not observed for the 2 vs. 3 discrimination.

The results obtained in this second experiment thus confirmed the importance of leaving stimuli visible at the moment of choice to guarantee motivation and guide the experimental subject's attention towards conspecifics. However, an interpretative limit that can be found in these results concerns the actual use of a proto-arithmetic in the 1 vs. 2 discrimination. In this condition, a conspecific is hidden behind the opaque barrier leaving the two remaining stimuli visible, one on each side. The animal could therefore join the larger group using only a concept of object permanence, knowing that "something" was hidden behind only one of the two panels (which coincides with the location of the larger group). This does not allow us to state that fish based their judgment on the numerical addition of quantities. The aim of the subsequent experiment was to evaluate whether the discriminative success in the 1 vs. 2 condition was actually attributable to arithmetic competence or not.

2.3. Experiment 3a: Addition and subtraction of conspecifics

This experiment tested whether the discriminative ability in the 1 vs. 2 condition (Experiment 2) was actually attributable to a proto-arithmetic ability rather than to a simpler object permanence of the stimulus hidden behind the panel. To do so, the 1 vs. 2 condition was carried out initially by hiding all the stimuli behind the opaque barriers (as in Experiment 1) and, before releasing the experimental animal, one conspecific from each hidden group was made visible again (ensuring a motivation in the subject's response, as shown in Experiment 2; see Fig. 3a).

2.3.1. Results and discussion

The GLMM analyses of the random effects did not show any influence on the statistical model (random intercept variance of the best fit equal to zero) and the order of movement of the stimuli (larger/smaller group moves as last), suggesting adopting the most suitable model that only considers the choices towards the major/minor group. The final best model estimate (logarithmic odds ratio) was 0.3957 ± 0.1275 . The binomial test showed a probability of success equal to 59.8 %, $p = 0.00213$ (95 % confidence intervals: 53.48–65.82), indicating a strong significant difference compared to chance towards the group with larger numbers (Fig. 3b).

The results obtained confirm that zebrafish can identify the largest group in the "1 vs. $1 + 1 = 2$ " condition, even if the stimuli were initially hidden behind the panel and then become partially visible again (one stimulus per group). The success of zebrafish in this condition is important, not only because it shows that the 1 vs. 2 discrimination is actually based on the ability to add single elements, but because it might suggest that fish can also manipulate subtractions in such a way of representing a concept of "empty" or "zero" set. Indeed, after observing "1 + 1" vs. "1" elements disappearing behind the panels, the reappearance of a conspecific from each screen would lead the zebrafish to elaborate that only behind one panel there is a conspecific left (i.e., the major group: $2 - 1 = 1$; the minor group: $1 - 1 = 0$). This hypothesis, however, remains to be validated. An alternative explanation could be that in the choice of the largest group, the animals did not consider how many fish actually remained behind the panel after the addition and subtraction of stimuli,

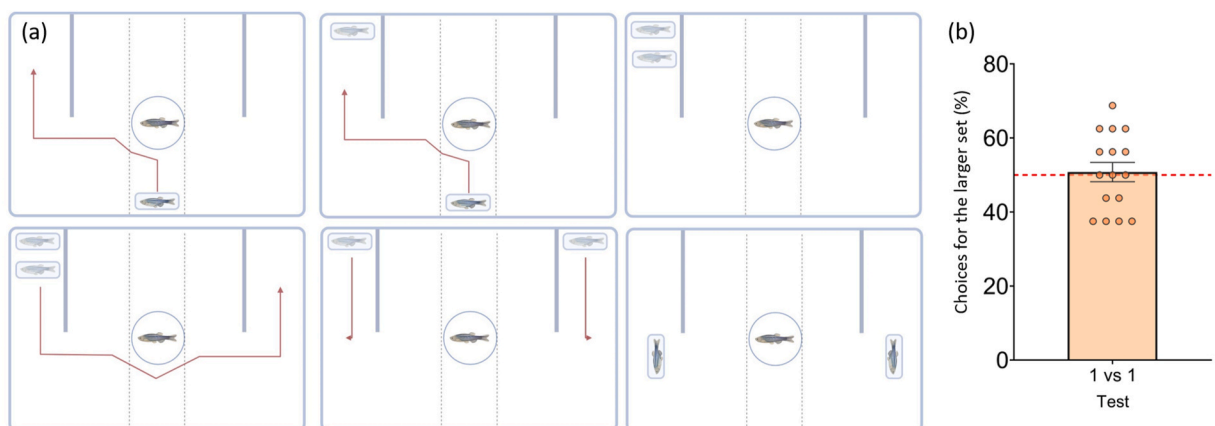


Fig. 4. (a) Schematic representation of conspecific positioning in the condition 1 vs. 1. Two conspecifics are hidden behind one of the two screens; then, a fish becomes visible again and is moved to the opposite screen. Finally, both fish are simultaneously moved becoming visible to the test subject. (b) Percentage of choice for the initially larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish.

being instead based their choice on the number of “path movements” made during the presentation of the stimuli (i.e., 2 movements for the larger group and only one for the smaller group). In order to evaluate which strategy was used by zebrafish, a control condition was carried out trying to create a conflict between the number of movements of conspecifics and the number of animals hidden behind the panels.

2.4. Experiment 3b: Control condition 1 vs. 1

This experiment represented a control of the Experiment 3 in order to evaluate whether zebrafish discrimination 1 vs. 2 involving additions and subtractions was based on the number of animals hidden behind the two panels instead of the number of path movements presented (ignoring what is actually behind the screens). The control condition steps were as follows: two sequential conspecifics were hidden behind one screen (creating a “1 + 1 = 2” scenario on one side), while on the other side, nothing happened; then, from the hidden group, a conspecific became visible again and was moved, retracing the initial path backward, behind the opaque screen on the opposite side (thus creating the scenario “2 - 1 = 1” vs. “1”, both not visible); finally, the stimuli were simultaneously made visible on both sides of the apparatus, effectively leaving two panels without hidden conspecifics (see Fig. 4a) and a discrimination condition 1 vs. 1.

From this condition three different outcomes could be predicted: 1) If the fish’s choice was based only on the number of episodes (number of presented movements) that occur on each of the two sides of the tank, then we should expect a preference for choosing the side on which the movements of the initial conspecifics take place; 2) if fish paid attention to what remained behind both screens, then they would have to choose the two sides randomly, given that on both sides there is the same number of conspecifics; 3) in the event that the final movement of the last animal from one screen to the other was particularly attractive for the experimental subject, we should predict an approach preference towards the side where the last movement has occurred.

2.4.1. Results and discussion

The GLMM did not show any interaction (random intercept variance of the best fit: 4×10^{-14}). The final best model estimate (logarithmic odds ratio) was 0.3125 ± 0.125 . The binomial test showed a random choice towards the two groups, with a probability of success equal to 50.8 %, $p = 0.85$ (95 % confidence intervals: 44.5–57.1) (Fig. 4b). To compare this control condition with the 1 vs. 2 condition in Experiment 3a, a novel model was created considering the two test conditions as a fixed effect. Results highlighted a significant relative difference between the tests ($\chi^2(1, N = 32) = 4.19, p = 0.041$). Post-hoc analyses highlighted a significant difference between the 1 vs. 1 control condition and the 1 vs. 2 condition ($Z = -2.041, p = 0.041$).

The outcome from this control condition shows that zebrafish have no preference for either group, suggesting that fish did not base their choice on the amount of movement observed on one side versus the other, but rather on the actual number of conspecifics present in the scene and hidden behind the screens. This hypothesis is supported by a significant difference between the present control condition and the previous 1 vs. 2 discriminative condition where (with the same number of movements and presentation of the stimuli) fish show a significant preference for the larger group. Overall, the results show not only that fish use proto-arithmetic on numerical quantities but also suggest a possible rudimentary knowledge of “empty or zero” set and subtraction (see also General Discussion section).

2.5. Experiment 4: Non-numerical variables control: total exposure time of individual conspecifics

This experiment includes a series of control conditions aiming at checking whether the preference for the larger group observed in the previous experiments could be influenced by the different amounts of time spent in moving the different groups of social stimuli. Since in all the previous experiments the motion of the conspecifics occurred at the same speed, we consequently had a greater total presentation time near the group with the largest numerosity. In this experiment, two experimental conditions were carried out, with the aim of controlling the overall time of moving the stimuli: in the first control experiment, the overall times presentation was balanced by moving the elements of the two groups with different speeds; in the second control experiment, the total number of movements was equalized by moving the conspecifics of the larger group in pairs (i.e., for each single movement of the minor group, two elements were moved simultaneously in the major group).

2.5.1. Experiment 4a: Time equalized by changing conspecifics velocity

Conditions and procedures were the same as in Experiment 2 (i.e., 1 vs. 2, 2 vs. 3, and 2 vs. 4), with the only difference of balancing the total movement time overall. The social stimuli, which were part of the smaller group, moved slower than those of the larger group. In this way, the sum of the individual time displacements for the major and minor groups was balanced. Since the movement was manually performed by the experimenter, a statistical analysis was conducted for each fish to ensure that any minimal differences between the movement times of the two groups ($\Delta t = \frac{\sum_{i=1}^{16} \text{time_bigger} - \sum_{i=1}^{16} \text{time_smaller}}{16}$) among all the session trials did not differ from chance level ($p > 0.05$).

2.5.1.1. Results and discussion. The GLMM analyses of the random effects (Fish ID) showed that they did not influence the statistical model (random intercept variance of the best fit: 4×10^{-14}), just as no significant difference was found relating to the type of Test, the order of movement of the stimuli and the interactions between the two factors, suggesting adopting the simplest model which takes into consideration only the choices towards the larger/smaller group, collapsing the test conditions together. The final best model

estimate (logarithmic odds ratio) was 0.2038 ± 0.073 . The binomial test, considering all test conditions together, showed a probability of success equal to 55.1 %, $p = 0.005$ (95 % confidence intervals: 49.54–62.04), indicating an overall significant difference compared to the chance level (Fig. 5).

When the overall stimuli movement time was balanced between the two groups (i.e., slowing down the speed of the group with the smaller number of conspecifics), zebrafish showed a general preference for the larger group, independently from the numerical condition (no difference between the 1 vs. 2, 2 vs. 3 and 2 vs. 4 conditions). This does not match with what was obtained in Experiment 2, where fish showed a spontaneous preference for the larger group only in the “1 vs. 2” condition and not in “2 vs. 3 and 2 vs. 4”. This might suggest that the conspecifics movement speed might turn out to be a strong attractive component that could even overcome the numerical information. However, given that the fastest movements always occur in the largest group, this might be tricky in the interpretation of what zebrafish are paying attention to (number or velocity). To better understand the role of numerical information and conspecifics’ velocity, it became necessary to run a further control condition aimed at creating a conflict between the two information, namely a decrease of speed occurring for the conspecifics forming the larger numerical group in order to disentangle which information did matter for zebrafish.

2.5.2. Experiment 4b: Quantity or velocity: what matters most?

The attempt to balance the overall conspecifics’ time exposure between the two groups revealed a side preference for the velocity in zebrafish. To understand how much velocity actually matters, a specific control condition relative to the 1 vs. 2 comparison was performed by proposing the opposite situation as done in Experiment 4a: the group in the smaller numerical group was moved at normal speed (movement duration: 9 seconds), while the larger numerical group was moved at half the speed. As a consequence, we end up with a conflict between numerical information and velocity (i.e., the smaller group is faster than the larger group). A missing preference for the larger group “1 + 1 would confirm a strong bias towards the velocity. This control was limited to the 1 vs. 2 comparison, given that the other higher numerical comparisons would have required a too-long overall presentation time.

2.5.2.1. Results and discussion. The GLMM did not show any interaction and the final best model estimate (logarithmic odds ratio) was -0.1566 ± 0.1254 . The binomial test showed a random choice towards the larger/smaller group, with a probability of success equal to 46.09 %, $p = 0.235$ (95 % confidence intervals: 39.87–52.41; Fig. 6).

This result showed that velocity attracted the attention and motivation to approach conspecifics with fast motility, probably due to the detriment of the effective attention towards the numerosness of the compared groups. However, the 1 vs. 2 condition does not differ from the chance level, suggesting that numerical information is not ignored.

Taken together, these series of control conditions show that, in a social context, the velocity of movement is an attracting factor that can distract zebrafish from the spontaneous use of quantity information. When the two pieces of information (i.e., magnitude and velocity) correlate positively, fish show increased general performance. Conversely, when magnitude and velocity do not correlate, fish perform at a chance level, meaning that they are influenced by both aspects. Summing up, from these experiments and particularly this last one, we can exclude that the overall exposure time is an important factor for zebrafish when performing a general spontaneous quantity discrimination of social companions. Even if the largest group’ movement employed more time, there was no preference towards it, namely the correlation between number and overall time exposure did not matter in fish’ choice.

2.5.3. Experiment 4c: Balance of the number of movements by presenting conspecifics of the larger group in pairs

This second control experiment was done to control whether zebrafish maintained a preference towards the larger group even when the number of presentations (i.e., the actual number of movements required to move the stimuli) was equalized. In order to do so, female conspecifics from the larger stimulus set were moved within the apparatus in pairs. In this way, the amount of movement performed by the experimenter was controlled and equalized in the two groups (Fig. 7a). Two control condition were performed here: 1 vs. 2 and 2 vs. 4.

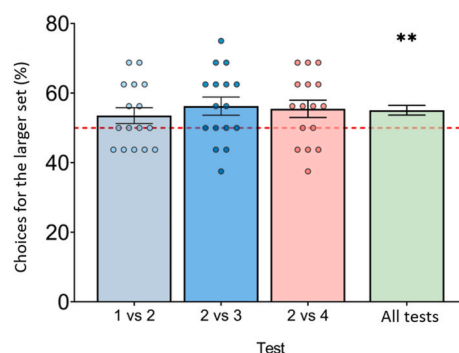


Fig. 5. Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]) in each discrimination condition (1 vs. 2, 2 vs. 3 and 2 vs. 4). Colored dots represent the individual performance of each fish. Significant departures from chance level (50 %) are shown by asterisks (** $p < 0.01$).

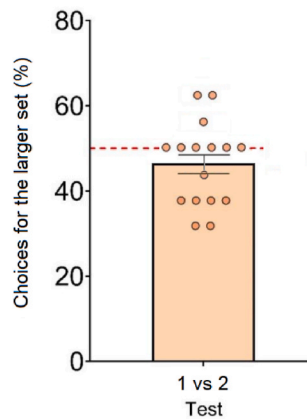


Fig. 6. Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish.

2.5.3.1. Results and discussion. No significant difference was found related to the type of Test, the order of movement of the stimuli, and the interactions between the two factors, suggesting adopting the simplest model, which takes into consideration only the choices towards the major/minor group, collapsing the test conditions together. The analysis of random effects showed a higher value (random intercept variance of the best fit: 0.035 ± 0.19) compared to all previous experimental conditions, suggesting that some animals may have different behavior compared to the other subjects in the sample (as can be seen from the distribution of points in the graphs in Fig. 7b). The binomial test showed no difference from chance (probability of success = 50.78 %, $p = 0.76$ (95 % confidence intervals: 46.36–55.19), confirming that zebrafish show no preference for one of the groups of conspecifics.

From the second control experiment, it has been clarified that in order to be able to observe an effective discriminative ability in zebrafish it is mandatory to have a single different number of movements/episodes. Indeed, when the total number of movements was equalized by moving the conspecifics of the larger group in pairs, fish showed a clear decline in performance, choosing randomly between the two groups. However, it is important to note that in both discriminative conditions (1 vs. 2 and 2 vs. 4) there are some

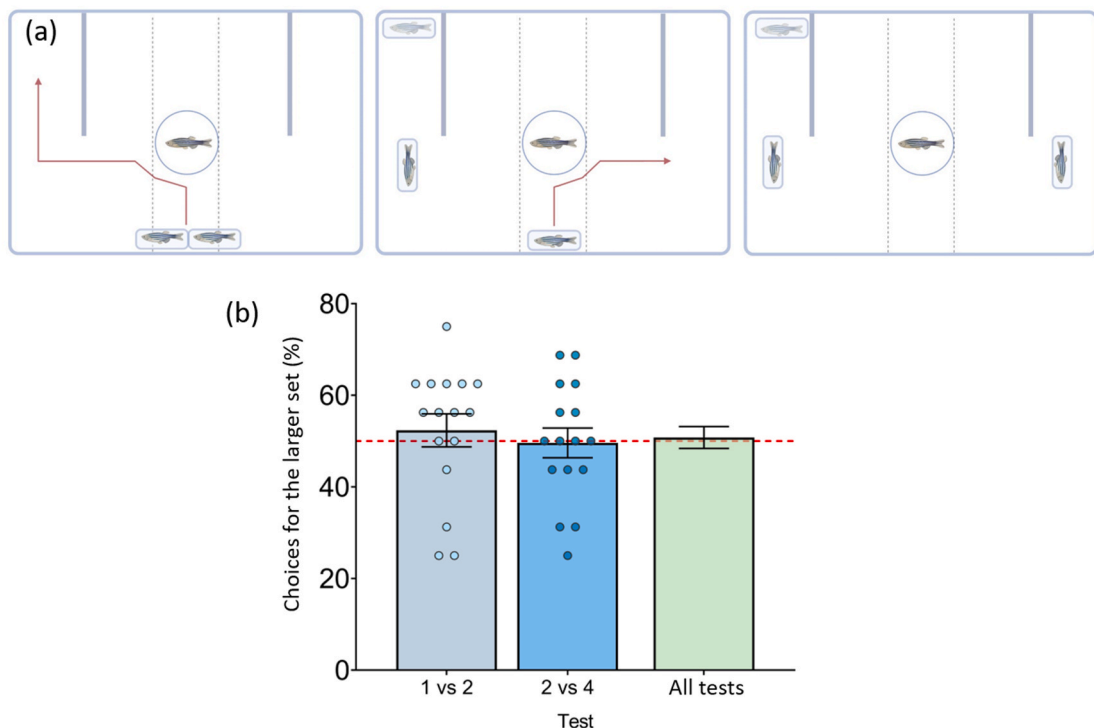


Fig. 7. (a) Schematic representation of the sequence of movements for pairwise positioning in the discrimination between 1 and 2 conspecifics. Conspecifics forming the larger and smaller groups were respectively moved in pairs and individually. (b) Percentage of choice for the larger set (mean \pm standard error of the mean [SEM]). Colored dots represent the individual performance of each fish.

animals with a clear preference for the smaller group; this tendency was not found in previous experiments and could lead to the hypothesis that pairwise movement could lead in some subjects an avoidance behavior.

3. Conclusions

This study explored zebrafish's use of proto-arithmetic in quantity discrimination by leveraging their shoaling behavior. Male zebrafish's ability to distinguish between groups of female conspecifics was observed, with varying numbers presented sequentially and hidden behind opaque panels. In the first experiment, zebrafish failed to reliably choose the larger group in a "1 vs. 2" comparison, possibly due to a lack of visual cues. A second experiment modified the procedure by leaving one stimulus visible for each group, which led to success in the "1 vs. 2" comparison but not in "2 vs. 3" or "2 vs. 4." This suggested that cognitive load and elapsed time between presentations might affect performance.

The third experiment confirmed that zebrafish could manage not only quantity addition but also subtraction in the "1 vs. 2" comparison and may have some understanding of the "empty set" or zero concept. A control experiment ruled out the possibility that the fish were simply tracking movement paths rather than actual group sizes. The fourth experiment adjusted for total presentation time by varying the speed of stimulus movement, revealing that faster-moving stimuli could override numerical information, with fish showing a general preference for velocity. However, when the number of movements was equalized, zebrafish performed at random, indicating that both numerical quantity and movement speed influence their choices.

The study suggests zebrafish might grasp rudimentary arithmetic concepts like addition, subtraction, and possibly the idea of zero or empty set.

4. General discussion

This study contributed to a better understanding of the numerical abilities of animals in proto-arithmetic tasks using zebrafish as a model. A primary result of our experiments is that zebrafish can distinguish between two groups of conspecifics with different numerical quantities, presented one by one in sequence and hidden behind occluding panels. This is the first evidence in zebrafish of proto-arithmetic abilities in a spontaneous choice paradigm and using biologically relevant stimuli. Schluessel and colleagues (2022) [21] demonstrated that two other species of cichlid and cartilaginous fish (*Pseudotropheus zebra* and *Potamotrygon motoro*) can learn numerical rules like "add 1" or "subtract 1" from a set of stimuli using extensive operant conditioning. It is important to note that in our study, fish exhibited their behavior without any learning procedure. The ability shown was based on their spontaneous tendency to aggregate in groups of conspecifics (shoaling behavior) and sexual competition.

In our study, the presentation of female conspecifics occurred one at a time, preventing the experimental male from having a simultaneous global view of social stimuli. This allowed us to conclude that any preference for the larger of the two groups was dictated by the ability to add individual events. Additionally, zebrafish' choice implies a memory recall of hidden objects and their positions, assessing not only discriminative quantity abilities but also working memory skills ranging from 9 to at least 40 s. Learning and remembering spatial information are essential for individual survival [37].

The results of the first experiment suggested that zebrafish might not be able to discriminate the larger of two groups of conspecifics sequentially presented and hidden behind opaque panels (not visible at the moment of choice) in the numerical condition "1 + 1 = 2 vs. 1". This could indicate a difficulty related to an overload of information for working memory. However, the results of the subsequent experiments contradicted this hypothesis, suggesting a lack of motivation to approach one of the two groups due to the absence of any visible conspecific at the moment of choice.

Results from Experiment 3 confirmed that the discriminative ability observed in the 1 vs. 2 condition was indeed attributable to proto-arithmetic capacity. This experiment introduced not only the addition of conspecifics (1 + 1 vs. 1) completely hidden behind the screens but also subtraction through the subsequent synchronized movement of two conspecifics (one per group), becoming visible to the test animal. This ruled out the possibility that the observed ability referred solely to the representation of a single non-visible object (an object permanence concept, which has been demonstrated in zebrafish by Sovrano and colleagues (2018) [38].

Moreover, Experiment 3 indicated that fish based their choice not so much on the quantity of movement of individuals observed during the presentation but rather on the actual numerosity of conspecifics present in the scene. Even when there was a conflict between the number of movements of conspecifics (greater on one side than the other) and the number of animals hidden behind the panels, the fish showed no preference, suggesting reliance not on the number of events proposed but actual knowledge of what was behind the panels. This leads us to hypothesize that zebrafish, through the ability to perform basic arithmetic operations (limited to "1 + 1," "1 - 1," and "2 - 1"), may also possess knowledge of the concept of an "empty set" or "zero".

The results obtained in this study suggest that zebrafish can spontaneously perform basic arithmetic operations, adding and subtracting individual quantities of conspecifics, limited to a few elements (maximum 2 elements per group in the discriminative condition 1 vs. 2). Zebrafish seem to have an actual numerical perception of elements hidden behind screens. However, to observe this ability, it is essential to have a sequential presentation of individual elements at a constant speed (Experiment 4).

The discriminative failure observed in some numerical conditions might be stated due to some biases rather than the measured variable [39]. Here, in each experiment, the stimuli presentation between the left-right sides of the apparatus was counterbalanced using a semi-random sequence, as was the order of stimulus presentation. Moreover, in half of the trials, the larger quantity was presented first, followed by the smaller quantity, and vice versa. This design ensures that any fish exhibiting a bias (such as a spontaneous preference for one side or a tendency to favor the first or last object presented) would perform at chance levels, preventing us from mistakenly interpreting a preference for one of the numerical groups. Therefore, we can conclude that any spontaneous

preference for one of the two numerical groups is solely due to the quantity or number of stimuli, rather than other biases. Furthermore, since we observe a significant preference in some experimental conditions (specifically in the 1 vs. 2 range), we can rule out the possibility that failures in other numerical conditions are due to biases affecting performance. Instead, these failures are likely attributable to limitations in quantitative discrimination.

As for the underlying system for performing arithmetic operations, be it an "Approximate Number System (ANS)" or "Object File System (OFS)", we do not have enough information to state which one they use. In this basic arithmetic, the representation of small numerical attributes and the mental manipulation of these representations in working memory might suggest that a precise object-tracking system is involved. However, this remains a hypothesis that needs to be validated, since we cannot exclude that an approximate system might be engaged.

The results provided for the first time evidence concerning the spontaneous use of proto-arithmetic abilities in numerical quantities in male zebrafish. In this study, the use of only males was preferred because of the strong attraction and competition among males for females that increases the test fish focus towards the stimuli. Moreover, data available in the literature on discrimination between different numerical groups of conspecifics is typically focused on males, giving us a baseline on the numerical ratios discriminated to interpret our results. Females show similar preferences for larger shoals though differences between sexes may be present related to boldness [40] and aggressive behavior [41]. The use of males in our study leaves open the possibility for future studies to investigate potential sex differences in numerical performance.

The zebrafish is a widespread model in developmental biology and genetics and is one of the best available models for studying the ontogenetic development of numerical cognition [42]. Thanks to the development of neurobiological techniques and the evidence collected on the neural basis of number in this species (see Messina et al. (2022) [5]), our results may provide behavioral techniques useful for studying the neural circuitry underpinning proto-arithmetic abilities in zebrafish.

5. Experimental model and subject details

A total of 192 adult male zebrafish were used for the spontaneous choice experiments involving 16 male fish for each condition as test subjects. To determine the required number of experimental subjects for the study, we conducted an a priori power analysis using R Software. Considering that each subject would participate in 16 binomial trials (a dichotomous choice between a larger group = 1 and a smaller group = 0), we calculated the sample size for a z-test for proportion with continuity correction (two-tailed), a statistical significance level (α) of 0.05, a power ($1 - \beta$) of 0.80 and a Cohen's h effect size of 0.5 (moderate), which showed a required sample size of 16 subjects per experimental group. Female zebrafish were used as target stimuli. The fish's total length was between 4 cm and 5 cm for both males and females. Males and females were distinguished through morphological characteristics, double-checked by the supplier and by expert laboratory personnel of the Animal House in order to guarantee high accuracy and safety.

Subjects were reared in an automated aquarium system (ZebTEC Benchtop, Tecniplast) specific for zebrafish housing, in 3.5L plastic tanks in isolated sex groups of 10 individuals each. Before the experiment, fish were transferred in groups of 10–15 subjects in 40 L tanks, and kept separated in groups based on sex. Each tank was enriched with gravel and vegetation and each aquarium contained a pump and filter system (Sera fil 60). Zebrafish were reared in standard conditions (26 °C, light/dark cycle of 14 h/10 h); feeding was provided two times per day using dry food (GVGMix SERA).

6. Method details

6.1. Apparatus

The experimental apparatus consisted of a rectangular plastic tank (36x23 × 20 cm) with white walls. Near the shorter sides of the tank, two white vertical Poliplak® panels (11x8.5 × 0.4 cm) were located. Each panel was placed 4.5 cm from the short side of the tank

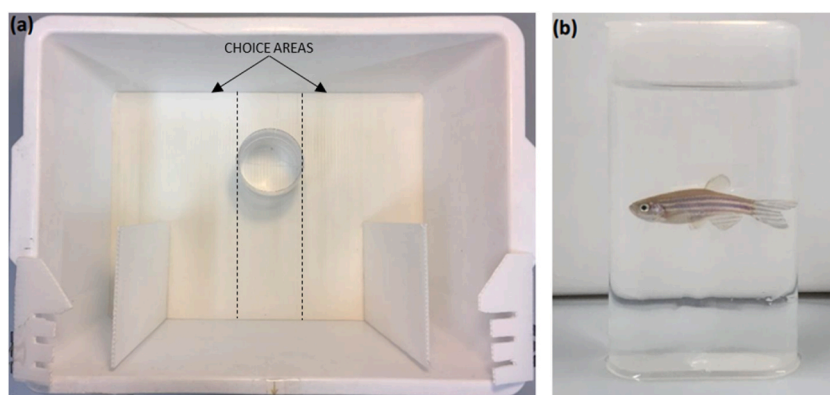


Fig. 8. (a) Photograph of the apparatus (b) Transparent box housing the female conspecific.

and was perpendicularly and adjacent to the same long side of the apparatus. These panels had the function of hiding two sectors of the apparatus, making it impossible to see behind them from the center (Fig. 8a). At the beginning of each trial, the test male was inserted into a transparent plastic cylinder (6.5 cm in diameter and 14.5 cm in height, 250 ml in water volume) and placed in the center of the apparatus in the neutral area. The cylinder was hooked to a pulley mechanism, through an invisible nylon thread. On the bottom of the tank, two lines were drawn to delineate a central neutral area (7×23 cm) and two choice areas (14.5×23 cm). These lines permit to have an objective assessment of the choices made by the subject during the test.

The water temperature was maintained at 26°C and the water was purified by a pump and filter system. Water physicochemical parameters were monitored and kept constant throughout all the experiments ($\text{pH} = 8$; Nitrite $\text{NO}_2 < 0.2$ mg/L; Ammonia $\text{NH}_3/\text{NH}_4 < 0.1$ mg/L Conductivity = $400\text{--}500$ μS). The same conditions were maintained both for the test and housing tank. During the experiment, the pump and filter system were removed and the water level in the experimental apparatus was kept at a height of 7.5 cm.

The test tank was in a darkened room, illuminated by a 60 W fluorescent lamp placed 80 cm above the tank. A webcam (Microsoft LifeCam Studio) was placed next to the lamp, to record the experiment from above.

6.2. Target stimuli

Adult female zebrafish were used as social stimuli for the behavioral procedure (we randomly selected 2 to 6 females for each session). Females were reared in a 40 L aquarium with gravel and vegetation.

The social stimuli were chosen since the attraction towards conspecifics is very strong and resistant to extinction. Furthermore, the motivation to reach the larger group of conspecifics remains stable for long periods and after many trials [22]. Several fish species, including zebrafish, show a typical gregarious behavior (shoaling) to receive protection from predators. Moreover, zebrafish females are very attractive to male subjects [43].

Female conspecifics were individually placed in transparent plastic boxes ($4.5 \times 8.5 \times 2$ cm; water volume 67 ml), which were equipped with a plexiglass handle (28 cm), that allowed them to move them easily without being seen by the experimental fish (see Fig. 8b). In this way, it was possible to present single conspecifics one at a time and to follow a defined trajectory. Each box was closed by a lid with a small opening, which allowed the flow of water, but prevented the animal from coming out. Each female was constantly monitored during the experiment and immediately replaced in case it showed stress-related behaviour (i.e., freezing, erratic movement, gasping, or rapid gill movement). Moreover, the water in each box was partially changed among trials maintaining an optimum quality level.

6.3. Familiarization phase

Prior to testing, two male fish were inserted in the experimental setup for approximately 1 h. During this time, they were free to move and feed to get familiar with the new environment. Subsequently, they were collected and placed in the aquarium from which they were kept separated from the other conspecifics.

6.4. General procedure

Before starting the test, a single fish was placed in the apparatus for 10 min to acclimate and food was provided. The food served to encourage shoaling behavior and to avoid competition between the animals. Thereafter, the female conspecifics used as attractive stimuli were randomly selected, making sure they were the same size as the test fish, and gently inserted into the small plastic box.

At the beginning of each trial, the test male was confined in a transparent plastic cylinder in the center of the apparatus, for 2 min. Subsequently, one female was introduced in the apparatus in front of the subject into the neutral area and was moved close to the cylinder to capture the attention of the test fish (i.e., the test fish must orient itself towards the moving stimulus), and then moved towards one of the two panels on the left or the right side. The same sequence was repeated one after another until all the female conspecifics were placed in the experimental setup, forming two groups. The order of presentation and the presentation procedure could undergo variations, depending on the experimental condition carried out, described in detail in each section.

Once all social stimuli were displaced, after 5 s the transparent cylinder was gently lifted from above. The fish was free to move and approach one of the two groups of conspecifics in the two opposite sectors for 2 min. A choice was considered valid when the fish entered with the entire body in one of the two areas identified by the black lines drawn on the floor. Once the trial was completed, the experimental subject was again confined into the cylinder, the conspecifics were removed from the apparatus, and, after a latency of 2 min, the next trial began.

If the experimental subject showed signs of stress and fear (freezing behavior) at the beginning of the procedure, the fish was excluded from the experiment and was replaced. Each animal performed a daily session of 16 consecutive trials. The position of the larger group of females was changed among trials and balanced between the right and left sides of the tank, following a semi-random sequence (e.g., dx-sx-dx-sx-dx-sx-sx) [44]. In this way, the largest group of female conspecifics was equally placed on the right and on the left during the 16 trials. Moreover, the order of entry of the two groups was also balanced in the session: in half of the trials, the larger group was first presented, followed by the smaller; for the other half vice-versa.

Regarding the amount of time required for moving each stimulus, the standard movement time for each conspecific was 9 s, except for the conditions in which the overall amount of exposure time between the two groups was balanced, thus requiring a different presentation time, according to the ratio between the numerosities proposed (e.g., in the 1 vs. 2 comparisons in Experiment 4a, the movements in the larger group required $9 + 9$ s, while in the smaller group there was a single movement lasting 18 s).

All tests were video recorded using a video camera placed above the apparatus, using Open Broadcaster Software (OBS) Studio.

6.5. Coding of behavior and statistical analyses

The coding of the animals' behavior took place during the session and was subsequently controlled by the video recordings; initially, the number of choices for the bigger and smaller groups of conspecifics was recorded. These lines permit to have an objective assessment of the choices made by the subject during the test. Thereafter, data were analyzed using R software (R-4.2.0), and choices for the larger and smaller numerosity were analyzed using a “generalized mixed model” (GLMM) fit by maximum likelihood (Laplace Approximation), binomial GLMM with a logit link. The best model was selected after a back-elimination procedure, removing interactions and factors iteratively, and comparing the different models based on AIC (Akaike information criterion) and BIC (Bayesian information criterion) information criteria which provide a measure of the distance between the model and the theoretical distribution of the data in relation to the number of parameters estimated. With reference to the model adopted, the probability ratio (Log odds ratio) was reported. A final binomial test was used to compare the distribution of the choices for the smaller and bigger groups of conspecifics when no factors were significantly contributing to the results.

In Experiment 4a, since the shift was manual, we additionally conducted a statistical analysis for each fish to ensure that any minimal differences between the movement times of the two groups ($\Delta t = \frac{\sum_{i=1}^{16} \text{time_bigger} - \sum_{i=1}^{16} \text{time_smaller}}{16}$) within the 16 trials, did not differ by chance ($p > 0.05$), using a paired-sample *t*-test.

7. Ethical regulation

The present research was carried out at the Animal Cognition and Neuroscience Laboratory (ACN Lab) of the CIMEC (Center for Mind/Brain Sciences), at the University of Trento (Italy). All husbandry and experimental procedures complied with European Legislation for the Protection of Animals used for Scientific Purposes (Directive 2010/63/EU) and were approved by the Scientific Committee on Animal Health and Animal Welfare (Organismo Preposto al Benessere Animale, OPBA) of the University of Trento and by the Italian Ministry of Health (Protocol n. 1111-2015-PR).

CRedit authorship contribution statement

Davide Potrich: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis. **Lorenza Montel:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Gionata Stancher:** Writing – review & editing, Methodology, Conceptualization. **Greta Baratti:** Writing – review & editing, Investigation. **Giorgio Vallortigara:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Valeria Anna Sovrano:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 833504 SPANUMBRA) and from Next Generation EU -European Union- PRIN 2022 PNRR (DD 1409 del 14/09/22) – PNRR – M4 – C2 – INV1.1 – PRIN – Title [The emergence of proto-arithmetic abilities with empty and non-empty sets] – project code [P2022TKY7B] – CUP [E53D23019640001].

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e40585>.

References

- [1] S. Dehaene, *The Number Sense*, Oxford University Press, Oxford, 1997.
- [2] G. Vallortigara, An animal's sense of number, in: A. Adams, J.W. Barmby, P. & Mesoudi (Eds.), *Nat. Dev. Math. Cross Discip. Perspect. Cogn. Learn. Cult.*, Routledge, New York, 2017, pp. 43–65.
- [3] A. Nieder, The adaptive value of numerical competence, *Trends Ecol. Evol.* 35 (2020) 605–617, <https://doi.org/10.1016/j.tree.2020.02.009>.
- [4] M. Bortot, L. Regolin, G. Vallortigara, A sense of number in invertebrates, *Biochem. Biophys. Res. Commun.* 564 (2021) 37–42, <https://doi.org/10.1016/j.bbrc.2020.11.039>.
- [5] A. Messina, D. Potrich, M. Perrino, E. Sheardown, M.E. Miletto Petrazzini, P. Luu, A. Nadtochiy, T.V. Truong, V.A. Sovrano, S.E. Fraser, C.H. Brennan, G. Vallortigara, Quantity as a fish views it: behavior and neurobiology, *Front. Neuroanat.* 16 (2022) 1–18, <https://doi.org/10.3389/fnana.2022.943504>.

- [6] B. Butterworth, *The Mathematical Brain*, Macmillan, London, 1999.
- [7] A. Nieder, S. Dehaene, Representation of number in the brain, *Annu. Rev. Neurosci.* 32 (2009) 185–208, <https://doi.org/10.1146/annurev.neuro.051508.135550>.
- [8] L. Feigenson, S. Dehaene, E. Spelke, Core systems of number, *Trends Cogn. Sci.* (2004), <https://doi.org/10.1016/j.tics.2004.05.002>.
- [9] D.C. Hyde, Two systems of non-symbolic numerical cognition, *Front. Hum. Neurosci.* (2011), <https://doi.org/10.3389/fnhum.2011.00150>.
- [10] S.R. Howard, A. Avargues-Weber, J.E. Garcia, A.D. Greentree, A.G. Dyer, Numerical cognition in honeybees enables addition and subtraction, *Sci. Adv.* (2019), <https://doi.org/10.1126/sciadv.aav0961>.
- [11] M. Panssar, The enculturated move from proto-arithmetic to arithmetic, *Front. Psychol.* 10 (2019), <https://doi.org/10.3389/fpsyg.2019.01454>.
- [12] K. Wynn, Addition and subtraction by human infants, *Nature* 358 (1992) 749–750, <https://doi.org/10.1038/358749a0>.
- [13] J. Christodoulou, A. Lac, D.S. Moore, Babies and math: a meta-analysis of infants' simple arithmetic competence, *Dev. Psychol.* (2017), <https://doi.org/10.1037/dev0000330>.
- [14] M.D. Hauser, P. Macneillage, M. Ware, Psychology numerical representations in primates, *Proc. Natl. Acad. Sci. U. S. A.* (1996), <https://doi.org/10.1073/pnas.93.4.1514>.
- [15] M.D. Hauser, F. Tsao, P. Garcia, E.S. Spelke, Evolutionary foundations of number: spontaneous representation of numerical magnitudes by cotton-top tamarins, *Proc. R. Soc. B Biol. Sci.* (2003), <https://doi.org/10.1098/rspb.2003.2414>.
- [16] M.J. Beran, M.M. Beran, Chimpanzees remember the results of one-by-one addition of food items to sets over extended time periods, *psychol. Sci.* 15 (2004) 94–99, <https://doi.org/10.1111/j.0963-7214.2004.01502004.x>.
- [17] I.M. Pepperberg, Grey parrot (*Psittacus erithacus*) numerical abilities: addition and further experiments on a zero-like concept, *J. Comp. Psychol.* 120 (2006) 1–11, <https://doi.org/10.1037/0735-7036.120.1.1>.
- [18] R. Rugani, L. Fontanari, E. Simoni, L. Regolin, G. Vallortigara, Arithmetic in newborn chicks, *Proc. R. Soc. B Biol. Sci.* 276 (2009) 2451–2460, <https://doi.org/10.1098/rspb.2009.0044>.
- [19] R.L. Rodríguez, R.D. Briceño, E. Briceño-Aguilar, G. Höbel, Nephila clavipes spiders (Araneae: nephilidae) keep track of captured prey counts: testing for a sense of numerosity in an orb-weaver, *Anim. Cogn.* 18 (2015) 307–314, <https://doi.org/10.1007/s10071-014-0801-9>.
- [20] R.E. West, R.J. Young, Do domestic dogs show any evidence of being able to count? *Anim. Cogn.* 5 (2002) 183–186, <https://doi.org/10.1007/s10071-002-0140-0>.
- [21] V. Schluessel, N. Kreuter, I.M. Gosemann, E. Schmidt, Cichlids and stingrays can add and subtract 'one' in the number space from one to five, *Sci. Rep.* 12 (2022) 1–11, <https://doi.org/10.1038/s41598-022-07552-2>.
- [22] R. Gerlai, Learning and memory in zebrafish (*Danio rerio*), *Methods Cell Biol.* (2016), <https://doi.org/10.1016/bs.mcb.2016.02.005>.
- [23] R. Spence, G. Gerlach, C. Lawrence, C. Smith, The behaviour and ecology of the zebrafish, *Danio rerio*, *Biol. Rev.* (2008), <https://doi.org/10.1111/j.1469-185X.2007.00030.x>.
- [24] A. Faccioli, R. Gerlai, Zebrafish shoaling, its behavioral and neurobiological mechanisms, and its alteration by embryonic alcohol exposure: a review, *Front. Behav. Neurosci.* (2020), <https://doi.org/10.3389/fnbeh.2020.572175>.
- [25] N. Ruhl, S.P. McRobert, The effect of sex and shoal size on shoaling behaviour in *Danio rerio*, *J. Fish. Biol.* 67 (2005) 1318–1326, <https://doi.org/10.1111/j.0022-1112.2005.00826.x>.
- [26] N.Y. Miller, R. Gerlai, Shoaling in zebrafish: what we don't know, *Rev. Neurosci.* (2011), <https://doi.org/10.1515/RNS.2011.004>.
- [27] P.S. Suriyampola, D.S. Shelton, R. Shukla, T. Roy, A. Bhat, E.P. Martins, Zebrafish social behavior in the wild, *Zebrafish* (2016), <https://doi.org/10.1089/zeb.2015.1159>.
- [28] M.B. Orger, G.G. de Polavieja, Zebrafish behavior: opportunities and challenges, *Annu. Rev. Neurosci.* 40 (2017) 125–147, <https://doi.org/10.1146/annurev-neuro-071714-033857>.
- [29] A.H. Groneberg, J.C. Marques, A.L. Martins, R. Díez del Corral, G.G. de Polavieja, M.B. Orger, Early-life social experience shapes social avoidance reactions in larval zebrafish, *Curr. Biol.* (2020), <https://doi.org/10.1016/j.cub.2020.07.088>.
- [30] E. Dreosti, G. Lopes, A.R. Kampff, S.W. Wilson, Development of social behavior in young zebrafish, *Front. Neural Circuits* (2015), <https://doi.org/10.3389/fncir.2015.00039>.
- [31] R.C. Hinz, G.G. De Polavieja, Ontogeny of collective behavior reveals a simple attraction rule, *Proc. Natl. Acad. Sci. U. S. A.* (2017), <https://doi.org/10.1073/pnas.1616926114>.
- [32] R.E. Engeszer, M.J. Ryan, D.M. Parichy, Learned social preference in zebrafish, *Curr. Biol.* (2004), <https://doi.org/10.1016/j.cub.2004.04.042>.
- [33] D. Potrich, V.A. Sovrano, G. Stancher, G. Vallortigara, Quantity discrimination by zebrafish (*Danio rerio*), *J. Comp. Psychol.* 129 (2015) 388–393, <https://doi.org/10.1037/com0000012>.
- [34] D. Seguin, R. Gerlai, Zebrafish prefer larger to smaller shoals: analysis of quantity estimation in a genetically tractable model organism, *Anim. Cogn.* 20 (2017) 813–821, <https://doi.org/10.1007/s10071-017-1102-x>.
- [35] L.M. Gómez-Laplaza, R. Gerlai, Spontaneous discrimination of small quantities: shoaling preferences in angelfish (*Pterophyllum scalare*), *Anim. Cogn.* 14 (2011) 565–574, <https://doi.org/10.1007/s10071-011-0392-7>.
- [36] C. Agrillo, M. Dadda, Discrimination of the larger shoal in the poeciliid fish *Girardinus falcatus*, *Ethol. Ecol. Evol.* (2007), <https://doi.org/10.1080/08927014.2007.9522574>.
- [37] M. Sison, R. Gerlai, Associative learning in zebrafish (*Danio rerio*) in the plus maze, *Behav. Brain Res.* (2010), <https://doi.org/10.1016/j.bbr.2009.09.043>.
- [38] V.A. Sovrano, G. Baratti, D. Potrich, A detour task in four species of fishes, *Front. Psychol.* 9 (2018) 1–7, <https://doi.org/10.3389/fpsyg.2018.02341>.
- [39] M. Davison, J.A. Nevin, Stimuli, reinforcers, and behavior: an integration, *J. Exp. Anal. Behav.* 71 (1999) 439–482, <https://doi.org/10.1901/jeab.1999.71-439>.
- [40] G.P. Way, A.L. Kiesel, N. Ruhl, J.L. Sneker, S.P. McRobert, Sex differences in a shoaling-boldness behavioral syndrome, but no link with aggression, *Behav. Processes* (2015), <https://doi.org/10.1016/j.beproc.2014.12.014>.
- [41] J.L. Sneker, E. Diestler, Sex differences in the expression of aggressive behavior and influences on social choice in zebrafish (*Danio rerio*), *Behav. Processes* (2023), <https://doi.org/10.1016/j.beproc.2023.104871>.
- [42] E. Sheardown, J.V. Torres-Perez, S. Anagianni, S.E. Fraser, G. Vallortigara, B. Butterworth, M.E. Miletto-Petrazzini, C.H. Brennan, Characterizing ontogeny of quantity discrimination in zebrafish, *Proc. R. Soc. B Biol. Sci.* (2022), <https://doi.org/10.1098/rspb.2021.2544>.
- [43] A. Nasiadka, M.D. Clark, Zebrafish breeding in the laboratory environment, *ILAR J.* (2012), <https://doi.org/10.1093/ilar.53.2.161>.
- [44] B.J. Fellows, Change stimulus sequences for discrimination tasks, *Psychol. Bull.* (1967), <https://doi.org/10.1037/h0024098>.