TITLE: Characterising ontogeny of numerosity discrimination in zebrafish reveals use of multiple, numerical and non- numerical mechanisms.

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Abstract

Non-symbolic number cognition based on an approximate sense of magnitude has been documented in adult zebrafish. Here we investigated the ontogeny of this ability using a group size preference task in juvenile zebrafish. Fish showed group size preference from 26 days post fertilization (dpf) and from 27 dpf fish reliably chose the larger group when presented with discrimination ratios from 1:8 to 2:3. When the ratio between the number of conspecifics in each group was maintained at 1:2, fish could discriminate between 1 vs. 2 individuals and 3 vs. 6, but not when given a choice between 2 vs. 4 individuals. These findings suggest that the systems involved in numerosity representation in fish do not operate separately from other cognitive mechanisms. Rather they suggest numerosity processing is the result of an interplay between attentional, cognitive and memory-related mechanisms that orchestrate numerical competence both in humans and animals. Our results emphasise the potential of the use of zebrafish to explore the genetic and neural processes underlying the ontogeny of number cognition.

Keywords: Zebrafish, Numerical cognition, Ontogeny, Quantity discrimination

Introduction:

The ontogeny of numerical cognition has been widely studied in humans (1,2) whereas research in animals has been restricted to a limited number of species: frogs (*Bombina orientalis*), fish (*Poecilia reticulata*) and domestic chicks (*Gallus gallus*), and dogs (*Canis lupus familiaris*). (3–6). Typically, in species other than chicks, numerical abilities change with developmental age until individuals reach adulthood. There are exceptions, however: for example, one day old guppies (*Poecilia reticulate*) can distinguish among numerical stimuli in a similar way to 40-day-old guppies (7).

Interestingly, guppies show no ratio effect (Weber's Law (8)) for contrasts between numerosities 1 to 4, but the familiar ratio effect for larger numerosities. Similar findings have been observed in human infants (9–13) suggesting the existence of conserved mechanisms of number discrimination, though little is known about either the neural or genetic bases of these abilities.

The observation that small number discrimination does not show a ratio effect but larger numerosity discrimination does has been taken as evidence for two mechanisms for assessing number (14,15): an object-tracking system (OTS) or subitizing system with an upper limit of 4 items, and an approximate number system (ANS) for numerosities greater than 4 (16–19). However, this has been challenged in both humans (20,21) and other species [e.g. Gallistel and Gelman (1992, 2000) (8,22) have proposed an accumulator mechanism, following Meck & Church (1983) (23), that operates across the numerical range]. Nonetheless, it is likely that numerosities larger than about 10 are estimated in a quite different way to smaller numerosities, perhaps using density x area or volume (24–26). However, whatever the mechanism, it may interact with other cognitive systems, such that the effects of ratio in a numerosity discrimination task could be affected by, for example, working memory or attention (27).

Although zebrafish are shoaling fish and demonstrate a tendency to associate with conspecifics from as early as 10 dpf (28) and a robust preference for conspecifics from

3 weeks (29), the ontogeny of number discrimination in this species has not been studied. Here we addressed this issue, as a prelude to investigating the genetic bases and neural circuitry of basic numerical capacities, using a group size preference assay. Group size preference assays build on the observation that species of shoaling fish spontaneously choose to associate with the larger shoal to avoid predation (30–32). This procedure has been widely used to investigate numerical competence in several fish species (e.g. guppy, mosquitofish, stickleback, red-tail splitfin) (5,33,34). As previous studies (35) suggest adult zebrafish utilise an approximate number system to represent both small and large numerosities, we presented juvenile fish with a choice between sets of individuals within the small (<4) number range as well as contrasts that span both small and large numbers.

Methods.

Subjects

All subjects and stimuli were juvenile zebrafish bred in the BSU at Queen Mary University of London. The fish were Tubingen (TU) wild type laboratory strain (36,37). In total ~2035 fish were used, ~1500 as stimulus fish, 535 as test fish (see individual experiments for N). At the time of the experiments the fish were 24-33 days post fertilization (dpf), ages chosen on the basis of previous studies suggesting zebrafish show group size preference from 3 weeks post fertilization (38,39). Prior to experiments fish were kept in large groups (40-50 fish per tank) of unknown sex in a recirculating system under standard rearing conditions (40). During rearing, fish were kept at a constant temperature of 28 +/- 2ºC, with a 14:10 light: dark cycle and fed twice a day with paramecium and Gemma 75/150 (Skretting, USA). Each subject was used only once. After testing all fish were returned to the facility and maintained as breeding stock.

Procedures:

Different versions of the group size preference (GSP) assay were used. Initial experiments assessing the ontogeny of number discrimination abilities employed an 'open shoal' assay as described by Bisazza et al. (2010) (5) (Fig 1a,b). In later experiments to assess the limits of zebrafish discrimination abilities, the apparatus was

refined to ensure equal spacing between stimuli fish as described in Gómez-Laplaza and Gerlai (2012, 2013) (33,41) (Fig1c,d,e). Fish were fed an hour before testing and all experiments were conducted between 10am and 13.00pm. Stimuli and test fish were matched for age and size. Fish behaviour was recorded using an overhead camera in the Daniovision larval tracking system (Noldus, UK). The video recordings were analysed using Ethovision software (Noldus, UK)

1. *Ontogeny of group size discrimination: Open shoal GSP.*

As previous work reports robust social preference for ages of 21 dpf and above (29), we investigated the ontogeny of GSP zebrafish from 21-30 dpf. As slight variation in developmental rates make exact staging difficult, we pooled across days matching for size. Stimuli were inserted into the stimulus compartments for 10 minutes prior to the test to control for movement, as more stationary larvae reduce the possibility of using motion as non-numerical cue. The first contrast tested was 1 vs. 3: three stimuli fish were placed behind the barrier on one side of the central compartment, and one fish behind the barrier on the other side of the central compartment. We subsequently assessed performance in 2 vs. 4, and 2 vs. 3 (N = 246 n= ~20 fish for each contrast and age group). The position of the larger group was counterbalanced across trials to control for any side bias. On the basis of results from a pilot experiment examining the time course of GSP in this apparatus (Supplementary Fig. 1), the test fish was introduced into the middle of the 'central compartment' and its behaviour over the next 5 min recorded. The time spent by the subject within a distance of 1 cm from the glass facing either of the stimulus compartments (choice area) was determined. Subjects that did not visit each choice area at least once were considered inactive and removed from analysis (34 out of 280). The output measure, the overall index, was calculated using the formula:

$$\frac{\textit{Time spent near larger group}}{\textit{Time spent near larger group} + \textit{Time spent near smaller group}}$$

The overall index gives us a measure of the preference for the larger group.

2: Limits of group size discrimination: Individual compartment GSP

A modified version of the apparatus and procedure described in Gómez-Laplaza and Gerlai (2012, 2013) (33,41) was used to assess the limits of juvenile zebrafish GSP. Stimulus fish were held in equal sized (1 x 1.5 x 1.5 cm) individual compartments as in Fig1c,d. Stimuli fish were introduced to their compartment with an opaque barrier between the stimulus fish and central compartment and allowed to habituate for 5 min. The test fish was introduced into the central compartment and allowed to explore the entire area for 5 min. After this time, the fish was restricted to a central domain using a clear plastic cylinder (diameter 2 cm) that was manually placed over the fish and used to usher it to the centre of the compartment (an etched circle indicated the centre of the central compartment). Once the test fish was in the centre, the barriers were lifted to reveal the stimulus fish. The test fish was allowed to view the stimulus fish for 1 min and then the holding cylinder was removed, and the behaviour of the test fish recorded over a 5 min period. We tested the ability of juvenile individuals to discriminate contrasts varying in difficulty: from an easy ratio (1 : 8) to a more difficult one (3 : 4). The following numerical contrasts were presented: 1 vs. 8, 1 vs. 3, 2 vs. 5, 2 vs. 4, 2 vs. 3, 3 vs. 4 and 2 vs. 2 (N = 78 n=10-15 fish for each contrast). For all contrasts tested, the left/right position of the larger group was counterbalanced. Contrasts were pseudo-randomised across the testing session on each day, and between days. 27-33 dpf fish were used.

3: Controlling for overall space

To test the hypothesis that fish were using the overall space occupied by the shoal as a discriminative cue rather than the number of conspecifics (5,42) we ran a control experiment where distance between the two most lateral fish was equal for both group sizes in 2 vs. 5, 2 vs. 4 and 2 vs. 3 discriminations. In this way, the outer stimuli fish on either side of the central compartment were positioned opposite each other (Fig 1c). We tested 30-33 dpf fish (N = 87, n=14-15 for each contrast) that were matched for age and size to stimulus fish

4: 1 : 2 ratio discrimination when crossing the small and large number range

To test the hypothesis that proximity to the limits of the subitizing range (3 items) influences number discrimination, we assessed the ability of juvenile fish to discriminate a 1:2 ratio in the small number range (1 vs. 2) and when the numerical distance was increased from 1 to 3 (1 : (1 vs. 2), 2 : (2 vs. 4) or 3 : (3 vs. 9)) and the total number of items increased from 3 (1 vs. 2) to 9 (3 vs. 6). We included the contrast 2 vs. 5 as a positive control. We tested 30-33 dpf fish (N = 90 n = 20-25 for each contrast) that were matched for age and size to stimulus fish

Statistics.

All data significantly deviated from normality (Kolmogorov–Smirnov test, $p < 0.05$). We therefore used non-parametric statistics for all our data sets. Kruskal-Wallis tests followed by pair wise comparisons with Bonferroni correction were carried out to assess significant differences between groups. One sample Wilcoxon Signed Ranks tests statistics were used to compare preference for the larger group to chance level (0.50). All statistical tests were two tailed. Analyses were carried out in SPSS 27.

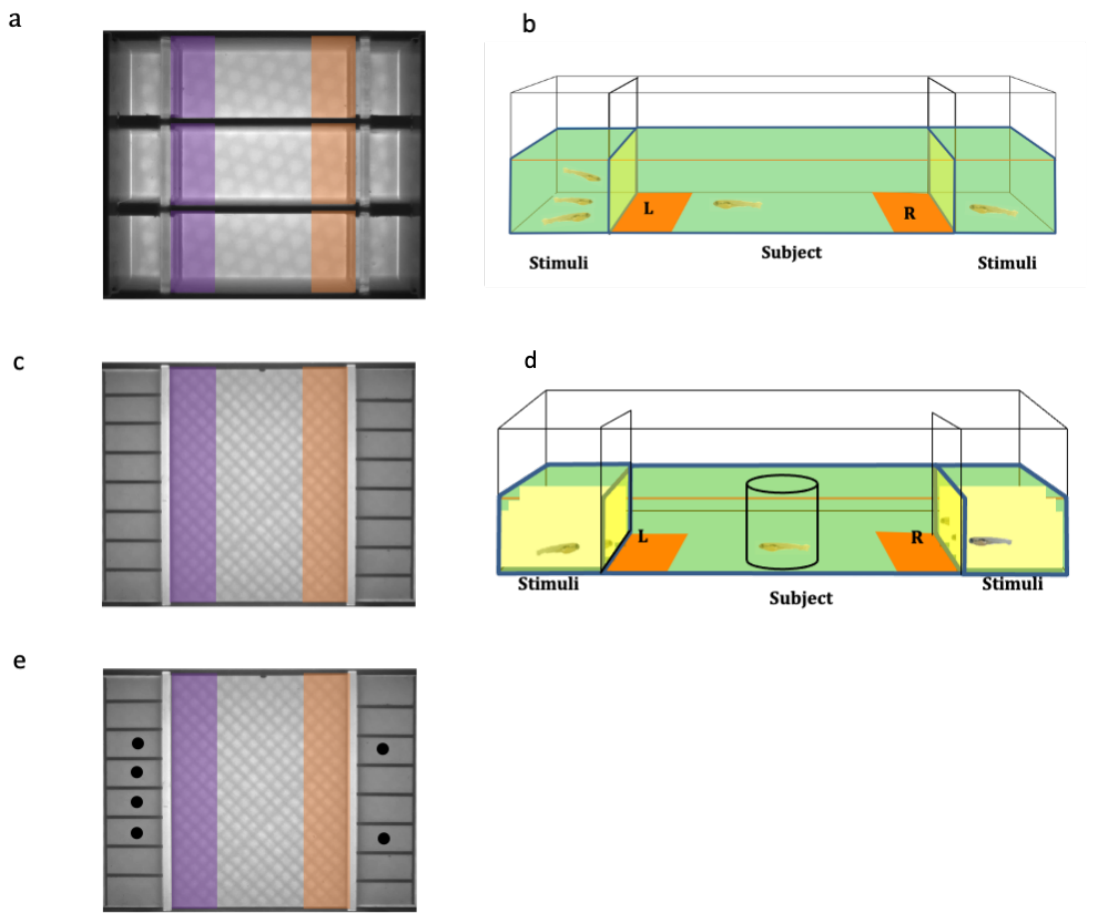

Figure 1 Screenshots (a, c) of apparatus and schematic representations of individual arenas (b, d). a. b, Open Shoal GSP. a). The set up consists of 3 arenas. Ethovision detects the presence of a subject in the left or right zones (indicated by coloured boxes), which sit 1cm in front of the stimulus windows. **b).** Each arena is composed of three adjoined compartments each of which consist of a central section flanked by two stimulus compartments. The central compartment is 6cm long and 2cm wide, with the 2cm sides being transparent for viewing the stimulus fish. The stimulus compartments are 2 cm long and 1cm wide to encourage the stimulus larvae to stay close to the viewing window for the test subject. All sides other than the stimulus window are opaque to avoid interaction between both stimulus and subject larvae in different arenas. **c). d). e). Individual compartment GSP.** The set up consists of a single arena. Ethovision detects the presence of a subject in the left or right zones (indicated by coloured boxes), which sit 1cm in front of the stimulus windows. **d).** The arena is divided into three adjoining compartments. The central compartment is 8cm long and 6cm wide, with the 8cm sides being clear for viewing the stimulus compartments. Each stimulus compartment is divided into eight 1.5 cm long and 1cm wide opaque sections. At the beginning of the trial the subject is held in a removable transparent cylinder with a 2cm diameter. A removable opaque barrier was present between the stimulus fish and test fish. **e).** Schematic of 'controlled space' conditions with the white dots representing the positions of the outermost zebrafish in a controlled 2 vs. 4 contrast.

Results

1. Ontogeny of group size discrimination: open shoal GSP.

When we first analysed the ontogeny of the preference for the larger group in a 1 vs. 3 contrast (Fig. 2) we found no overall difference in preference for the larger shoal between different ages ($\chi^2(5) = 5.674, p = 0.129$). However, one sample Wilcoxon signed rank tests showed there was a significant preference for the larger shoal at 26-28 dpf ($Z = 2.575, p < 0.01$) and 30 dpf ($Z = 2.688, p < 0.01$) but not at earlier ages. When additional contrasts were tested (2 vs. 4, 2 vs. 3), no significant effect of age was observed for either 2 vs. 4 ($\chi^2(3) = 0.843, p = 0.839$) or 2 vs. 3 ($\chi^2(3) = 3.184, p = 0.364$). One sample Wilcoxon signed rank tests showed there were no significant preferences for the larger shoal compared to chance at any age in these two contrasts (all p values > 0.05).

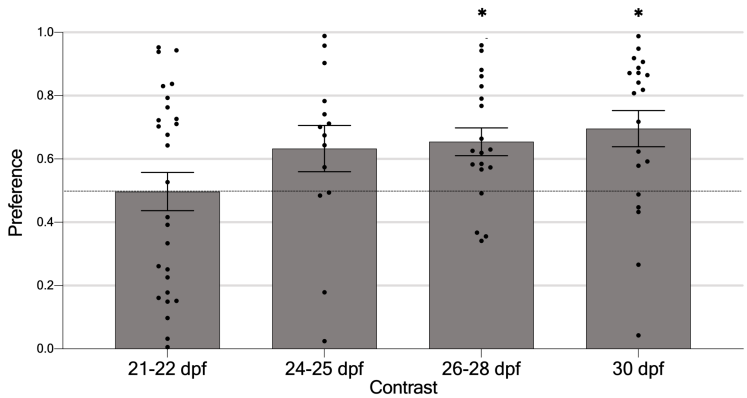

Figure 2 Ontogeny of group size preference in Open Shoal GSP. The zebrafish show an increase in preference for the larger group with age in a 1 vs. 3 contrast, with significant preference at 26-28 and 30 dpf. Dashed line indicates chance level, asterisks indicate significant difference to chance with $p < 0.05$.

2. Limits of group size discrimination.

We investigated the limits of discrimination in juvenile zebrafish. There was a significant difference in the time spent near the larger group between the contrasts ($\chi^2(6) = 18.505, p < 0.01$). Post hoc pairwise comparisons revealed there was a significant difference between 1 vs. 8 and 2 vs. 2 ($p = 0.05$). One sample Wilcoxon

signed rank tests showed a statistically significant preference for the larger group when compared to chance in 1 vs. 8 ($Z = 2.81$, $p < 0.01$), 1 vs. 3 ($Z = 2.51$, $p = 0.012$). 2 vs. 5 showed a non- significant trend in preference for the larger group when compared to chance ($Z = 1.762$, $p = 0.07$) (Figure 3a).

3. Controlling overall space

When the overall space occupied by the shoal was controlled, a Kruskal-Wallis test (for 'controlled space' condition) showed there was a main effect of space condition ($\chi^2(1) = 4.296$, $p = 0.038$) such that when the overall space occupied by the shoal was controlled fish spent significantly less time with the larger group. One sample Wilcoxon signed rank tests showed a statistically significant preference for the larger group when compared to chance only in the non-controlled groups for 2 vs. 5 ($Z = 2.198$, $p = 0.028$) and 2 vs. 3 ($Z = 2.419$, $p = 0.016$) but not 2 vs. 4 (Figure 3b).

4. *1 : 2 ratio discrimination when crossing the small and large number range*

A significant difference in preference between contrasts was found ($\chi^2(3) = 8.631$, $p = 0.035$) (1 vs. 2, 2 vs. 4, 2 vs. 5, 3 vs. 6). Post hoc pairwise comparisons revealed there was a borderline significant difference in the preference for the larger group between 1 vs. 2 and 2 vs. 4 ($p < 0.07$) and 3 vs. 6 and 2 vs. 4 ($p < 0.07$), with 1 vs. 2 and 3 vs. 6 showing greater preference for the larger group than 2 vs. 4. Juvenile fish successfully discriminated 1 vs. 2 ($Z = 2.738$, $p < 0.01$), 3 vs. 6 ($Z = 2.487$, $p = 0.013$) and 2 vs. 5 ($Z = 2.223$, p = 0.026) but not 2 vs. 4 (Figure 3c).

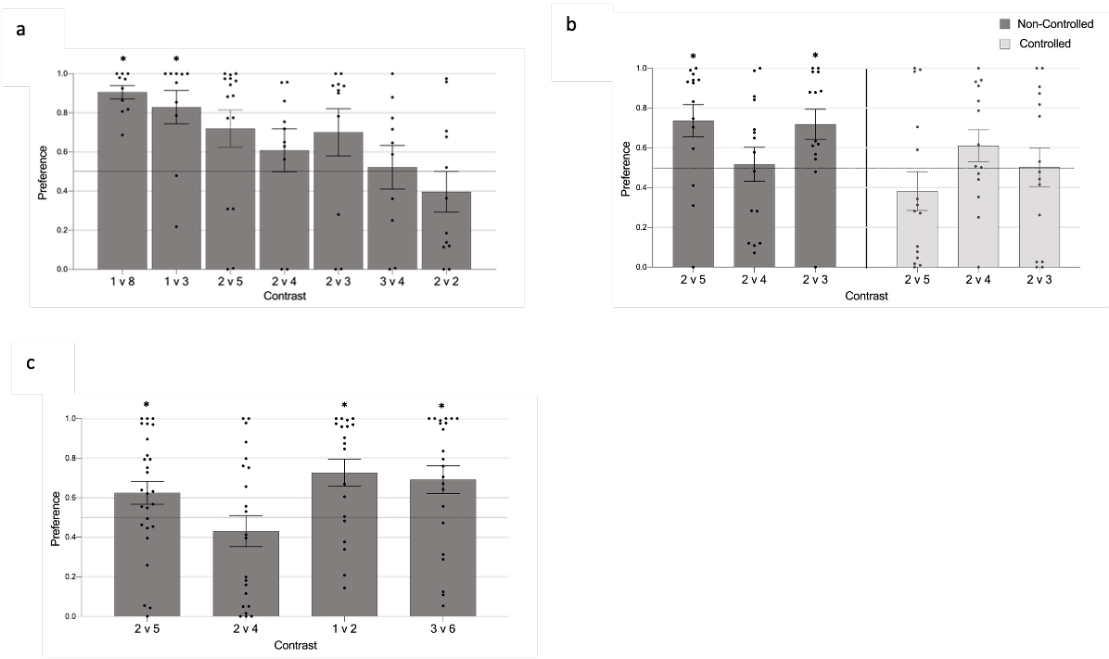

Figure 3. Investigating quantity discrimination. a). Discrimination abilities. The fish successfully discriminate 1 vs. 8 and 1 vs. 3. The control 2 vs. 2 shows no preference. **b). Controlling for overall group space.** Without controlling for overall area (light grey columns), the fish show a significant preference for the larger group in both the 2 vs. 5 and 2 vs. 3 controls. Controlling for overall area results in absence of preference in all the contrasts **c). Investigating 1: 2 ratio.** Fish successfully discriminate 2 vs. 5, 1 vs. 2 and 3 vs. 6 but not 2 vs. 4. Dashed lines indicate chance level, asterisks indicate significant difference to chance with $p < 0.05$.

Discussion

Despite the adaptive value of numerical competence (43), research has mainly focused on adults with developmental studies limited to a few species. Here we investigated quantitative skills of juvenile zebrafish using a 'group size preference' task in which the fish had to choose between two groups of conspecifics differing in number.

Several fish species have been found to join the larger shoal when exploring a novel and potentially dangerous environment to reduce predation risk (34). However, despite adult zebrafish forming tight multimember groups, social preference only becomes robust at the third week (29).

When assessing the ontogeny of group size preference from 3 weeks of age, we observed a developmental change in the preference to approach the larger group of conspecifics as zebrafish consistently selected 3 social companions over 1 only from the fourth week of life. The one-week delay observed here in comparison with Dreosti et al. (2015) (29) may be ascribed to methodological differences as those authors presented a choice between 0 vs. 3 or 0 vs. 1 fish: a condition in which the subject could choose between motion vs. absence of motion. It is possible that movement of conspecifics acted as a 'pop-out' and facilitated choice of the compartment with living stimuli. In the present study a motion cue was available on both sides of the apparatus possibly making the task more difficult as the fish cannot rely on the presence/absence of motion to make a choice.

In order to select the safer group in our assay fish had to assess a difference in quantity, although not necessarily a numerical difference; previous studies show that fish can rely on non-numerical cues, including motion, to discriminate shoal size (44,45). 26-30 dpf fish selected the larger group when given a choice of 1 vs. 3 conspecifics, however, when more difficult contrasts were used (2 vs. 4 and 2 vs. 3), none of the ages tested chose the larger shoal. The finding that 26-30 dpf fish could only discriminate 1 vs. 3 individuals is consistent with findings in humans, guppies, tadpoles and dogs where, although possessing some number sense at birth, numerical acuity increases across development (5–7,46,47). Hence, it is possible that zebrafish possess some numerical skills at an early age, but they are not mature enough to discriminate comparisons of increased difficulty. Alternatively, the use of the 'open shoal' assay may have influenced results. In this open shoal assay stimulus fish are free to swim with each other. This raises the possibility that visual occlusion of stimulus fish could have led to continuous changes of the numerical comparisons during the trials (e. g. 2 vs. 4 could become 2 vs. 2) thus making the discriminations more challenging.

In order to exclude this possibility, when examining the acuity of juvenile fish numerosity, we modified the experimental apparatus and procedure such that stimulus fish were held in individual compartments that allowed little movement so that the subject fish could look at both groups from an equidistant position before making a choice. These changes not only let the subject have a global view of the two groups

371 (33,41) but also minimized differences in stimulus fish activity, thus controlling for fish
372 movement and orientation.
373
374 Despite finding an effect of the contrasts presented, significant differences in choice
375 accuracy were only seen when comparing results of the 1 vs. 8 contrast with the 2 vs.
376 2 condition, a condition included to exclude possible side bias within the apparatus.
377 Fish showed a strong preference for the larger group only in the easiest contrasts (1 vs.
378 8 and 1 vs. 3), although we observed a tendency to join the larger group in 2 vs. 5 and
379 2 vs. 3. One possibility for the lack of significance in these latter contrasts may be the
380 relatively small sample size used as fish successfully discriminated 2 vs. 5 and 2 vs. 3
381 in subsequent experiments where a greater sample size was employed.
382
383 Interestingly, when we visually examined fish preference distribution within each
384 contrast, in all cases but 1 vs. 8 some individuals spent more time close to the smaller
385 shoal. This variability may reflect inter-individual differences in behaviour as a result
386 of differences in personality traits such as sociability (48,49) and boldness (50–52).
387 Such inter-individual differences may be predicted to have greater impact when small
388 sample sizes are used.
389
390 The numerosity of a set covaries with several continuous quantities, such as the overall
391 space occupied by the shoals, that can be used as a proxy to estimate which set is
392 larger/smaller (45,53). When we controlled for the overall space, juvenile zebrafish did
393 not choose the larger group of conspecifics. However, successful discrimination was
394 observed in 2 vs. 3 and 2 vs. 5 but not in 2 vs. 4 when overall space was not controlled.
395 These results seem to suggest that the overall space occupied may have driven the
396 choice for the larger group. However, the fact that fish consistently failed to select the
397 larger group when presented a choice between 2 and 4 individuals suggests this is not
398 the whole story, as 4 fish occupy twice the space. Even though other continuous
399 variables may also contribute to fish numerosity discrimination, use of continuous
400 variables alone would not predict the failure of this contrast. These data therefore
401 support the hypothesis that zebrafish use numerical as well as non-numerical
402 information to represent number as in other species including humans. Our results
403 where fish did not choose the larger group of 2 vs. 4 even when multiple visual cues

were available (24,54) suggests there is some aspect of the underlying mechanisms that renders this specific comparison challenging.

Previous behavioural, neuroimaging and electrophysiological studies provided evidence of the existence of two distinct systems for processing numerosities in humans, the OTS and ANS (14,27,55). Some argue that the ANS alone is recruited for processing discriminations over the whole numerical range. Others, instead, argue that representations of small numbers (< 4) are carried out by the OTS whereas the ANS is activated solely for representation of large numbers (> 4) (14,55). Evidence supporting the latter hypothesis comes from studies showing that 12-14-month-old infants discriminated between 1 vs. 3 and 2 vs. 3 objects but failed in 2 vs. 4 and 1 vs. 4 contrasts, despite being ratios they are able to discriminate in the large number range (9,13,56–58). This impairment in the discrimination of quantities that spanned the two systems has been suggested to indicate that the OTS and the ANS separately process small and large number and that their representations are difficult to merge. Nevertheless, conflicting results have been reported regarding the ability to compare small and large numbers provided the ratio between them was large enough (59) in agreement with the existence of a unique non-verbal numerical system (60).

In animals, despite most of the studies suggesting that a single mechanism such as the ANS is involved in processing both small and large numbers (61–63), there is also evidence of two different systems (27,60,61). For instance, adult redtail splitfin succeeded when presented with 1 vs. 4, 2 vs. 4, 1 vs. 2 and 2 vs. 3 comparisons but failed in a 3 vs. 4 comparison. These data support the idea of a continuity in processing small and large numbers. A similar conclusion was drawn by Potrich et al. 2015 (35) who reported ratio dependent discriminations of large and small shoals in adult zebrafish and no difficulty in comparing 2 vs. 4 fish. Conversely, two separate numerical systems have been suggested to operate in angelfish (66,67). However, contrasting results on the ability to discriminate quantities that crossed the small/large boundary (1 vs. 4) led to the suggestion that numerical processing may be context-dependent (42,68,69). Finally, evidence showing that guppies successfully discriminated large numbers only (5 vs. 10) and small numbers only (3 vs. 4) but failed to compare small and large numbers (3 vs. 5), unless the distance between them was

generous enough (e. g. 3 vs. 6) suggested that two systems were present, as in humans (70).

In our study, when zebrafish were presented with three comparisons with the same ratio (0.5), they discriminated 1 vs. 2, 3 vs. 6 but not 2 vs. 4, thus violating Weber's law, which states that numerical discriminations are based on the ratio between the represented magnitudes (22). Hence, accuracy should decrease as the ratio increases, but not when the ratio is constant. Furthermore, discrimination did not follow the distance effect as successful discrimination was observed for a distance of 1 or 3 units but not 2. Finally, according to numerical magnitude/distance effects, accuracy should decrease when the numerical distance is held constant but the numerical size of the contrasts increases, a condition we did not observe here as there was no difference in performance between 2 vs. 5 and 3 vs. 6. All together, these findings suggest that numerosity representation in juvenile zebrafish did not adhere to the principles of the ANS. However, juvenile zebrafishes' ability to distinguish groups containing a large number of conspecifics (i.e. 6), suggests that they are equipped with a mechanism for processing them.

Cordes and Brannon (2009) (59) proposed two hypotheses to explain infants' ability to discriminate small (< 4) versus large (> 4) numbers when a sufficiently large ratio is given (59). According to the "noise hypothesis", infants may first represent small sets through the OTS and large sets through the ANS and then convert object files into approximate magnitudes to compare the two quantities. In this scenario, the conversion from one representation to the other generates noise which increases with the number of items in the small set and necessitates a greater ratio for successful discrimination. Alternatively, according to the "threshold hypothesis", infants may represent both small and large sets through the ANS, but below a ratio threshold and within the small number range the representations through the OTS trump the ANS because they are more precise and reliable. However, when the ratio between small and large numbers exceeds a threshold, small/large comparisons can be performed solely using the ANS. In this case, success should be consistently predicted by the same ratio. Neither of these hypotheses can explain our finding that juvenile fish can discriminate 2 vs. 5 and 3 vs. 6 but not 2 vs. 4 items as the noise hypothesis predicts that 3 vs. 6 creates more noise

than 2 vs. 4, and the threshold hypothesis predicts that as 2 vs. 4 and 3 vs. 6 has the same ratio, there would be no difference in performance.

However, a third hypothesis suggests that the two systems are not differently specialized for small and large numbers but rather that attentional constraints and working memory determine if a set of items is represented as discrete individuals by the OTS or as an approximate numerical magnitude by the ANS (27). Here, the OTS would not be a mechanism specific for numerosity processing, rather it would be a more general system that allows parallel individuation of multiple objects that are stored in the working memory. Numerosity would then be indirectly inferred due to one-to-one correspondence between each real item and its mental representation (19). This mechanism seems to depend on attention as the number of items we can store in memory is related to the ability we have to attend to them (71–73) suggesting that the ANS, that operates over the entire range of numerosity, is supplemented by an attention-based system, the OTS, specific for small number, that does not operate under demanding attentional load conditions (27,72,74). Our data are consistent with this latter hypothesis. The break-down of performance at 2 vs. 4 in all the experiments suggests that the maximum number of items juvenile zebrafish can attend and retain in memory is 5. When the groups exceed this limit, fish appear able to represent numbers as quantities through the ANS, provided that a minimum numerical distance is held, which seems to be 3 (2 vs. 5 and 3 vs. 6). However, when a contrast is close to the upper limit, we speculate that the fish may still try to focus on each item but then fail to keep track of them and be unable to activate the ANS as the difference between the two shoals is too small, thus leading to a failure to discriminate. Hence, here we suggest that the systems involved in numerosity representation do not operate separately from other cognitive mechanisms, rather that numerosity processing is the result of an interplay among attentional, cognitive and memory-related mechanisms that orchestrate numerical competence both in humans and animals. The findings that adult zebrafish can memorize and discriminate 2 vs. 4 conspecifics (35) indicate that the mechanisms underlying numerical representation change over development as it is the case for humans. Future research investigating when this ability develops and if changes in attentional capacity and working memory allow such comparison to be made effectively can provide new insight into the complex mechanisms at the basis of numerical competence.

Despite the zebrafish being a powerful model in the field of translational neuroscience research to investigate molecular mechanisms underlying neuropsychiatric disorders (75–77), a limited number of behavioural assays are available to investigate related cognitive deficits. In humans, impairments of non-numerical abilities have been widely described in dyscalculia (a number and arithmetic learning disorder) (15,78,79) and evidence of familial aggregation suggests a genetic component in the evolution of this disorder (80,81). In recent years, the existence of an evolutionarily conserved non-verbal numerical system among vertebrates with a possible shared genetic basis has been suggested (14,34). In this context, the high degree of genetic homology with humans (82) makes zebrafish extremely useful to investigate the genetic mechanisms of numerical competence and their role in dyscalculia. Our study contributes to this aim as we provide a behavioural tool for the assessment of numerical abilities at an early age that can be employed for rapid screening of mutant lines for candidate genes potentially associated with poor numeracy.

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Ethics: All animal procedures in this study were reviewed by the QMUL ethics committee (AWERB) and conducted in accordance with the Animals (Scientific Procedures) Act, 1986 and Home Office Licenses.

Data availability statement: Supporting data files will uploaded to dryad on acceptance

Author Contributions: CHB, MEMP, BB, SEF, GV conceived and designed the study. ES, MMP, JVPT, SA, RR conducted experiments. ES, MEMP, BB, GV, CHB analysed and interpreted data. All authors contributed to writing the manuscript.

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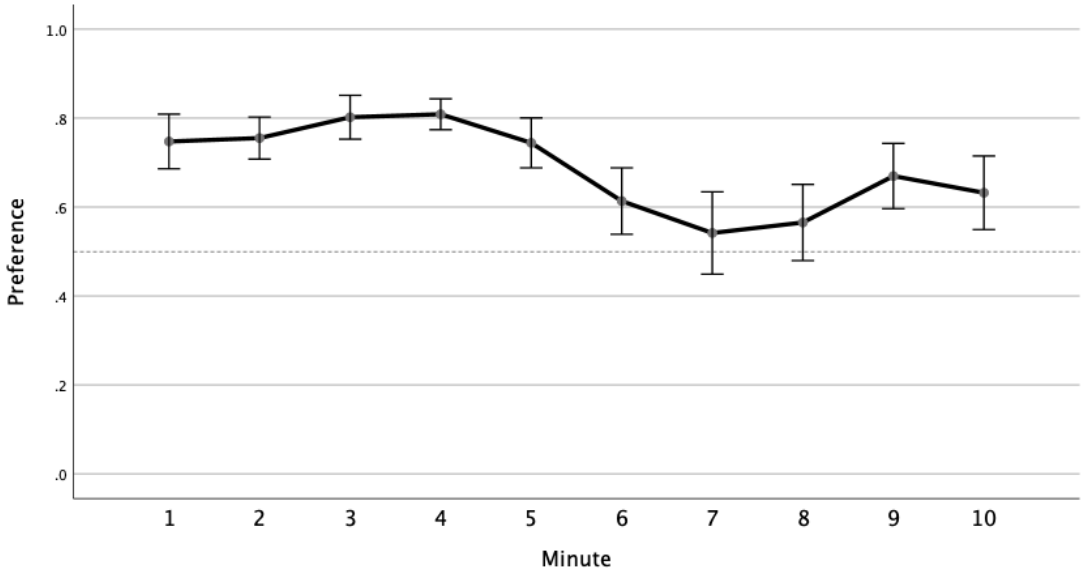
SUPPLEMENTARY:



Time course of preference. The zebrafish show a preference for the larger group in a 1 vs. 3 contrast in minutes 0-5 but not in minutes 5-10 with a significant reduction in the preference between the two time periods. Dashed line indicates chance, asterisk indicates significant difference to chance with $p < 0.05$.