

RESEARCH ARTICLE

Non-visual numerical discrimination in a blind cavefish (*Phreatichthys andruzzii*)

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ABSTRACT

Over a decade of comparative studies, researchers have found that rudimentary numerical abilities are widespread among vertebrates. While experiments in mammals and birds have employed a variety of stimuli (visual, auditory and tactile), all fish studies involved visual stimuli and it is unknown whether fish can process numbers in other sensory modalities. To fill this gap, we studied numerical abilities in *Phreatichthys andruzzii*, a blind cave-dwelling species that evolved in the phreatic layer of the Somalia desert. Fish were trained to receive a food reward to discriminate between two groups of objects placed in opposite positions of their home tank. In Experiment 1, subjects learned to discriminate between two and six objects, with stimuli not controlled for non-numerical continuous variables that co-vary with numbers, such as total area occupied by stimuli or density. In Experiment 2, the discrimination was two versus four, with half of the stimuli controlled for continuous quantities and half not controlled for continuous quantities. The subjects discriminated only the latter condition, indicating that they spontaneously used non-numerical information, as other vertebrates tested in similar experiments. In Experiments 3 and 4, cavefish trained from the beginning only with stimuli controlled for continuous quantities proved able to learn the discrimination of quantities based on the sole numerical information. However, their numerical acuity was lower than that reported in other teleost fish tested with visual stimuli.

KEY WORDS: Numerical cognition, Approximate number system, ANS, Core number system

INTRODUCTION

The study of numerical abilities in non-human species represents one of the main issues of research in cognitive ethology. The capacity to discriminate discrete quantities permits animals to solve a wide range of problems in their natural environment, such as selecting the larger quantity of food items to optimize food intake (Garland et al., 2012) or joining the larger group of social companions to minimize the risk of being captured by predators (Hager and Helfman, 1991). To date, rudimentary numerical abilities have been described in non-human primates (Beran et al., 2012; Vonk, 2014), other mammals (Baker et al., 2011; Kilian et al., 2003; Vonk and Beran, 2012; Panteleeva et al., 2013), birds (Garland et al., 2012; Hunt et al., 2008; Roberts, 2005), fish (Agrillo et al., 2012b; Buckingham et al., 2007; Gómez-Laplaza and Gerlai, 2011a) and even invertebrates (Pahl et al., 2013).

Most experiments on numerical cognition have utilized visual stimuli, such as groups of geometric figures presented on a monitor (e.g. Brannon and Terrace, 1998; Agrillo et al., 2013) or groups of food items visible on different plates (e.g. Baker et al., 2011; Perdue et al., 2012). However, non-visual stimuli have been often used as well. Non-verbal numerical abilities in our species have been reported with both auditory (Agrillo et al., 2010b; Lipton and Spelke, 2003) and tactile (Krause et al., 2013; Plaisier et al., 2009) stimuli with apparently no difference in number acuity across the sensory modalities involved (Feigenson, 2007). Concerning non-human primates, chimpanzees (*Pan troglodytes*) were shown to compare auditory quantities in a way that closely resembled how they compared visual quantities (Beran, 2012). Tamarins (*Saguinus oedipus*) tested with the habituation–dishabituation paradigm (Xu and Spelke, 2000) proved able to discriminate between two and three auditory tones (Hauser et al., 2002), a performance similar to that obtained with visual stimuli (Hauser et al., 2000). In rats (*Rattus norvegicus*), both auditory (Davis and Albert, 1986) and tactile information (Davis et al., 1989) have been used. Olfactory information has been investigated in a recent study on invertebrates: Carazo et al. (Carazo et al., 2009) found that male beetles (*Tenebrio molitor*) spontaneously discriminate sources of odors reflecting one versus four and one versus three females when prevented from seeing them. Evidence for an intermodal transfer of numerical information [considered to be significant evidence of abstract modality-independent numerical representation (see Davis and Perusse, 1988)] has been reported in trained rats (e.g. Meck and Church, 1983), and a recent study showed that untrained macaques (*Macaca mulatta*) also spontaneously match the number of voices they hear to the number of faces presented on a monitor, showing that their numerical abilities are unfettered by sensory modality (Jordan et al., 2008).

Evidence of a similar numerical acuity for visual and non-visual stimuli has led some authors to hypothesize the existence of a single, evolutionarily ancient, modality-independent numerical system (Beran, 2008; Feigenson, 2007; Jordan et al., 2008). Indeed, the existence in humans of a single magnitude system for processing numerical, temporal and spatial information has been hypothesized (Walsh, 2003). Indirect evidence also supports the existence of a common magnitude system in both mammals and birds (reviewed in Agrillo and Miletto Petrazzini, 2013).

In numerical cognition experiments, it is fundamental to assess whether an animal is actually using the numerical information or is instead using other types of information provided by the stimulus. In fact, the numerosity of a set of objects co-varies with other continuous properties, such as the cumulative surface occupied by the objects and their density, or the total area occupied by the sets. Such variables, commonly called continuous quantities, can be used by human and non-human animals to indirectly estimate the numerosity of a set (Gebuis and Reynvoet, 2012a; Gebuis and Reynvoet, 2012b; Gómez-Laplaza and Gerlai, 2012; Gómez-

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Laplaza and Gerlai, 2013). For instance, in a recent study (Pisa and Agrillo, 2009), trained domestic cats (*Felis silvestris catus*) were found to discriminate between two and three dots. In the training phase, stimuli were not controlled for continuous quantities, and both numbers and continuous quantities could be used to select the reinforced numerosity. In the subsequent test phase, stimuli were controlled for cumulative surface area: the cats' performance dropped to chance level, suggesting that they spontaneously learned to use continuous quantities instead of numbers. As discriminations based on numerical information or continuous quantities often yield similar results (Feigenson, 2007), controlled experiments are necessary to assess the relative role of discrete (numerical) and continuous quantities.

Recently, the work of cognitive ethologists has expanded to encompass fish species. Spontaneous choice tests (Dadda et al., 2009; Piffer et al., 2012; Gómez-Laplaza and Gerlai, 2011b) and training procedures (Agrillo et al., 2010a; Agrillo et al., 2011) showed that fish are capable of processing both small and large numbers with a performance similar to that described in mammals and birds tested with similar paradigms (Barnard et al., 2013; Hunt et al., 2008; Revkin et al., 2008). In particular, guppies (*Poecilia reticulata*) and mosquitofish (*Gambusia holbrooki*) show the ability to discriminate between shoals differing by one up to four items (one versus two, two versus three, and three versus four); larger quantities can also be discriminated provided that the numerical ratio between the smaller and the larger quantity is at least 0.50 [i.e. eight versus 16 (Agrillo et al., 2008; Agrillo et al., 2012a)]. Both species can also be trained to discriminate between two and three geometric figures when continuous quantities are controlled for (Agrillo et al., 2009; Agrillo et al., 2012b). An interspecific study investigating five distantly related species showed that guppies, zebrafish (*Danio rerio*), angelfish (*Pterophyllum scalare*), Siamese fighting fish (*Betta splendens*) and redtail splitfin (*Xenotoca eiseni*) display a similar performance in the same numerical tasks, suggesting the existence of shared numerical systems among teleost fish (Agrillo et al., 2012b).

So far, all fish studies have been restricted to the visual modality, leaving open the question of whether fish are able to process numerical information in non-visual modalities, as previously reported in mammals and birds. In the present study, we investigated non-visual numerical abilities of fish. Specifically, we tested a blind cavefish (*Phreatichthys andruzzii* Vinciguerra 1924) that has evolved for two million years in complete darkness. This species shows eye degeneration, making it a proper model to investigate the capacity to process numbers and continuous quantities in a fish brain using a non-visual modality. In a previous study, this cavefish was found to learn discrimination between three-dimensional objects with different shapes (Sguanci et al., 2010). There are no data available on the sensory modality (or a combination of different modalities) used by *P. andruzzii* to detect objects present in their

environment. In other hypogean fish there is evidence that information acquired by the lateral line system is used to memorize landmarks and form cognitive maps (De Perera, 2004; Teyke, 1989), and a recent study (Sguanci et al., 2010) suggests that this may be the main modality used by *P. andruzzii* as well.

Unlike the majority of other fish species of a similar size, *P. andruzzii* is extremely slow-growing and has a very low recruitment rate (Berti and Messana, 2010). A very limited number of individuals are available in worldwide laboratories. For this reason, we adopted an experimental design commonly used to test numerical abilities in mammals and birds (Jaakkola et al., 2005; Pepperberg, 2006; Vonk, 2014). In these studies, a small number of individuals are tested, sometimes even a single subject, under the assumption that if at least one individual can achieve the task, the species is equipped with neuro-cognitive systems able to potentially support the resolution of the task (Pepperberg and Brezinsky, 1991). We accordingly trained a limited number of individuals for each experiment and conclusions were drawn by the analyses of individual performance instead of group analyses.

We performed four experiments. We firstly assessed (Experiment 1) whether cavefish show the ability to discriminate between two quantities by presenting a 0.33 ratio (two versus six objects), a ratio that is known to be easily discriminated by teleost fish in the visual modality (Agrillo et al., 2010a; Agrillo et al., 2012a; Gómez-Laplaza and Gerlai, 2012). At this stage, a control for continuous quantities was not included. Subsequently, we asked whether cavefish can discriminate a higher numerical ratio, 0.50 (two versus four objects), both when numerical information and continuous quantities were available and when numerical information only was available (Experiment 2). As cavefish in this experiment could discriminate using continuous quantities but not using the sole numerical information, we asked whether cavefish could be trained to use numerical information by allowing them to use numerical cues from the start of the experiment; to assess their numerical acuity, two numerical ratios were presented: 0.50 (two versus four, Experiment 3) and 0.67 (two versus three, Experiment 4).

RESULTS

Experiment 1: discrimination of two versus six objects

Two fish were singly housed in a circular tank (Fig. 1). During training, at intervals, two stimuli (two versus six vertical sticks) were introduced at opposite ends and food was provided near the larger quantity. The proportion of time spent near the positive stimulus in probe trials without reward was taken as a measure of discrimination performance. In this experiment, stimuli were not controlled for continuous quantities that co-vary with number (e.g. total volume, density and overall space occupied by the two arrays). As a consequence, both number and continuous quantities could be used to solve the task.

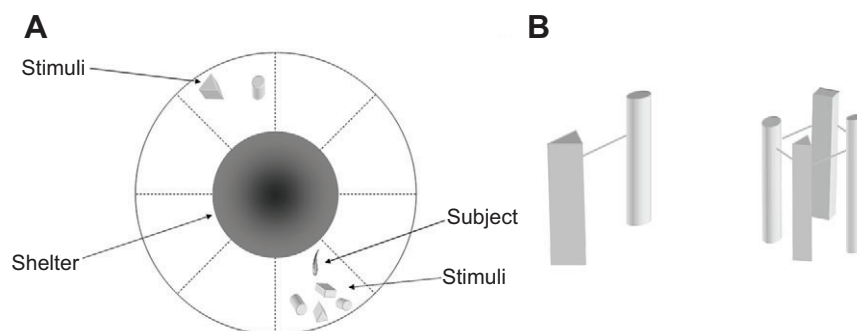


Fig. 1. Experimental apparatus and stimuli. (A) A circular tank was adopted to prevent the possibility of using geometrical information of the environment. The tank was divided into eight equal sectors: stimuli were placed in opposite sectors and food was provided only in correspondence of the reinforced quantity. A shelter was provided in the middle of the tank. (B) Stimuli were composed of groups of sticks differing in numerosity. In the figure, we depicted a schematic representation of a two versus four contrast.

Both subjects discriminated between the two quantities (subject 1: mean \pm s.d.=0.605 \pm 0.210, one-sample Wilcoxon signed rank test, $P=0.037$; subject 2: 0.579 \pm 0.139, $P=0.023$). A comparison of the performance on the first five trials versus the last five trials indicated no difference in performance over time (subject 1: accuracy in the first five trials: 0.689 \pm 0.195, last five trials: 0.616 \pm 0.314, Wilcoxon test: $Z=-0.730$, $P=0.465$; subject 2: first five trials: 0.665 \pm 0.140, last 5 trials: 0.566 \pm 0.136, $Z=-0.944$, $P=0.345$).

Experiment 2: discrimination of two versus four objects

At the end of Experiment 1, the same two fish were tested with a new series of stimuli using the same apparatus and procedure. Subjects were presented with a more difficult numerical ratio (0.50, two versus four objects). In half of the trials, stimuli were not controlled for continuous quantities ('number + continuous quantities' condition) as in Experiment 1. In the other half of trials, stimuli were controlled for continuous quantities and therefore only numerical information was available.

Both subjects discriminated between the two quantities (subject 1: 0.560 \pm 0.112, one-sample Wilcoxon signed rank test, $P=0.005$; subject 2: 0.557 \pm 0.156, $P=0.016$). When the performance was analyzed separately for the type of stimuli (number only / number + continuous quantities), we found a significant discrimination only in the condition in which both number and continuous quantities were simultaneously available (subject 1: number only, $P=0.167$; number + continuous quantities, $P=0.010$; subject 2: number only, $P=0.526$; number + continuous quantities, $P=0.037$; Fig. 2). No difference in performance over time was observed in the condition successfully discriminated by fish (subject 1: first five trials: 0.568 \pm 0.105, last five trials: 0.486 \pm 0.042, Wilcoxon test: $Z=-1.753$, $P=0.080$; subject 2: first five trials: 0.498 \pm 0.247, last five trials: 0.667 \pm 0.103, $Z=-0.944$, $P=0.345$).

Experiment 3: discrimination of two versus four objects using numerical information only

To verify whether cavefish were capable of solving the task of the previous experiment using numerical information only, four new subjects were trained from the beginning with stimuli controlled for all continuous quantities. Two fish were trained toward the larger numerosity as positive and two toward the smaller numerosity as positive.

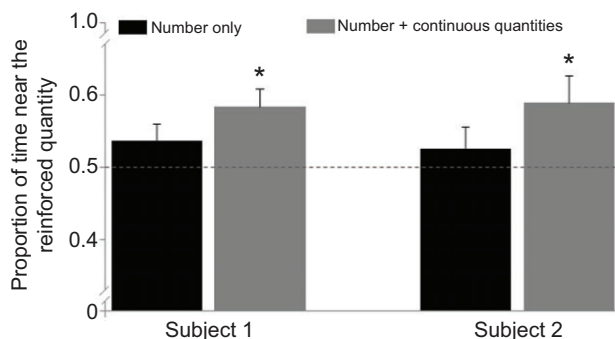


Fig. 2. Results of Experiment 2. Accuracy (proportion of time near the reinforced quantity) is plotted against the type of stimuli (number only, number + continuous quantities) separately for each subject. Both subjects successfully discriminated when number and continuous quantities were simultaneously available, while no choice was found in the number-only condition. Asterisks denote a significant departure from chance level ($P<0.05$). Horizontal dashed line indicates chance level. Error bars indicate s.e.m.

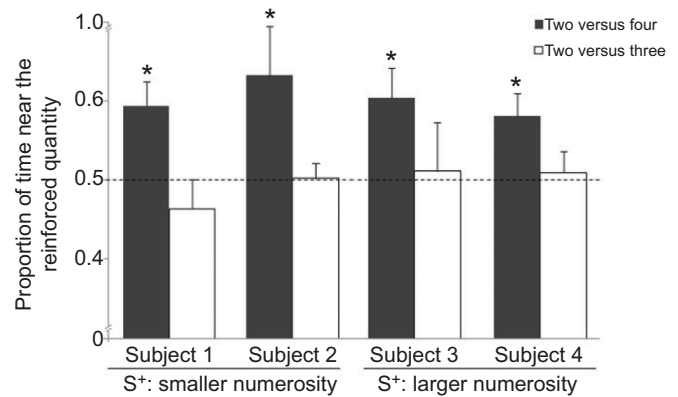


Fig. 3. Results of Experiments 3 and 4. Accuracy (proportion of time near the reinforced quantity) is plotted against the numerical contrasts (two versus four, two versus three) separately for each subject. Subjects 1 and 2 were trained with the smaller numerosity as positive (S+); subjects 3 and 4 were trained with the larger numerosity as positive. All subjects successfully discriminated two versus four, while no discrimination was found when the numerical distance was reduced (two versus three). Horizontal dashed line indicates chance level. Error bars indicate s.e.m.

Subject 1 (smaller numerosity as positive)

A significant discrimination was found (one-sample Wilcoxon signed rank test, $P=0.007$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.645 \pm 0.210, 85%: 0.523 \pm 0.139, 100%: 0.584 \pm 0.147, Friedman test, $\lambda_2=2.000$, $P=0.368$). A significant discrimination was also found when we analyzed the performance in all test trials that were 100% matched for volume (0.601 \pm 0.112, one-sample Wilcoxon signed rank test, $P=0.008$). No difference in performance over time was observed (first five trials: 0.546 \pm 0.099, last five trials: 0.627 \pm 0.133, Wilcoxon test: $Z=-0.730$, $P=0.465$).

Subject 2 (smaller numerosity as positive)

A significant discrimination was found ($P=0.001$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.636 \pm 0.419, 85%: 0.611 \pm 0.374, 100%: 0.680 \pm 0.300, $\lambda_2=0.933$, $P=0.627$). A significant discrimination was also found when we analyzed the performance in all test trials that were 100% matched for volume (0.640 \pm 0.210, $P=0.015$). No difference in performance over time was observed (first five trials: 0.660 \pm 0.092, last five trials: 0.662 \pm 0.200, $Z=-0.135$, $P=0.893$).

Subject 3 (larger numerosity as positive)

A significant discrimination was found ($P=0.044$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.575 \pm 0.254, 85%: 0.560 \pm 0.199, 100%: 0.648 \pm 0.098, $\lambda_2=0.500$, $P=0.779$). A significant discrimination was also found when we analyzed the performance in all test trials that were 100% matched for volume (0.628 \pm 0.147, $P=0.041$). No difference in performance over time was observed (first five trials: 0.538 \pm 0.410, last five trials: 0.648 \pm 0.153, $Z=-0.674$, $P=0.500$).

Subject 4 (larger numerosity as positive)

A significant discrimination was found ($P=0.023$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of

the stimulus type (70%: 0.560 ± 0.090 , 85%: 0.518 ± 0.103 , 100%: 0.636 ± 0.200 , $\lambda_2 = 1.500$, $P = 0.472$). A significant discrimination was also found when we analyzed the performance in all test trials that were 100% matched for volume (0.610 ± 0.047 , $P = 0.034$). No difference in performance over time was observed (first five trials: 0.498 ± 0.156 , last five trials: 0.638 ± 0.114 , $Z = -1.214$, $P = 0.225$). As in this and in the previous experiments the performance did not vary across the test trials, fish are likely to have already learned the discrimination in the preceding phases.

Experiment 4: discrimination of two versus three objects using numerical information only

As other teleosts proved able to discriminate more difficult ratios [e.g. 0.67 ratio, two versus three objects (see Agrillo et al., 2009; Agrillo et al., 2011)], the same four cavefish of Experiment 3 were tested in a two versus three discrimination with stimuli controlled for all continuous quantities.

Subject 1 (smaller numerosity as positive)

No discrimination was found (one-sample Wilcoxon signed rank test, $P = 0.351$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.628 ± 0.098 , 85%: 0.465 ± 0.223 , 100%: 0.539 ± 0.150 , Friedman test, $\lambda_2 = 2.300$, $P = 0.078$).

Subject 2 (smaller numerosity as positive)

No discrimination was found ($P = 0.709$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.463 ± 0.066 , 85%: 0.503 ± 0.104 , 100%: 0.487 ± 0.087 , $\lambda_2 = 0$, $P = 1.0$).

Subject 3 (larger numerosity as positive)

No discrimination was found ($P = 0.940$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.520 ± 0.098 , 85%: 0.517 ± 0.399 , 100%: 0.556 ± 0.373 , $\lambda_2 = 2.000$, $P = 0.368$).

Subject 4 (larger numerosity as positive)

No discrimination was found ($P = 0.627$; Fig. 3). When different types of volume control (100, 85 and 70%) were alternated in the first 12 test trials, the accuracy did not vary as a function of the stimulus type (70%: 0.517 ± 0.134 , 85%: 0.500 ± 0.169 , 100%: 0.490 ± 0.206 , $\lambda_2 = 0.500$, $P = 0.779$).

DISCUSSION

Many species of fish can discriminate subtle differences among different amounts of items presented in the visual domain. In the present work, we examined whether fish can accomplish such a task in a non-visual modality by studying numerical discrimination in a blind cavefish.

In the first experiment, we tested cavefish with a numerical task (0.33 ratio, two versus six objects) that is easily discriminated by teleost fish in the visual modality (Agrillo et al., 2012a; Agrillo et al., 2012b). After 26 trials in which subjects were progressively trained to receive food in proximity of the larger set of objects, both subjects significantly selected the larger set. An unresolved issue in this study is the identification of the sensory modality involved in numerical discrimination. Because *P. andruzzii* shows a complete anophthalmia (Berti et al., 2001), only the use of another sensory

modality can be advocated to explain the results we obtained. The two main candidates are the sense of touch and the lateral line system. The latter in particular is generally well developed in blind cavefish compared with the sighted surface form and plays a crucial role in different behavioral responses (Yoshizawa et al., 2010; Yoshizawa and Jeffery, 2011). In *P. andruzzii*, the lateral line system is already well developed in the larvae and the neuromasts increase in number and size both in the anterior (head) and posterior (trunk) lateral line with age (Dezfuli et al., 2009). Based on behavioral observations, Sguanci and collaborators (Sguanci et al., 2010), who have studied discrimination of three-dimensional shapes in *Phreatichthys*, suggest that this task might be accomplished mainly through the lateral line system.

Results of Experiment 1 may provide also indirect insights into the debate surrounding the existence of one or two systems of numerical representation in non-human animals. To date, some studies support the existence of a single approximate system of numerical representation, commonly referred to as the 'approximate number system'. This system has no upper limit but is subject to a ratio limit in accordance with Weber's law (Judge et al., 2005; Merritt et al., 2009; Smith et al., 2003; Ward and Smuts, 2007). Other studies, however, hypothesized the existence of a separate system, called the 'object tracking system', which would be co-opted to precisely enumerate small quantities, usually up to three to four items (Gómez-Laplaza and Gerlai, 2011b; Hauser et al., 2000; Murofushi, 1997; Piffer et al., 2012). One prediction of the two-system hypothesis is that a discrimination between numbers across the small (less than three or four)/large (more than four) number boundary would determine a conflict between the two types of representation, leading to a reduced performance (Feigenson and Carey, 2005). Our study was not expressly designed to test these hypotheses. However, the finding of Experiment 1 that cavefish proved able to make a direct comparison between small and large numbers (two versus six objects) is in line with recent studies in mammals (Cantlon and Brannon, 2007; Hanus and Call, 2007) and birds (Rugani et al., 2009; Rugani et al., 2013a; Rugani et al., 2013b) that showed no decrease in performance in small versus large number comparisons and thus is more in agreement with the single-system hypothesis.

The result of Experiment 1 alone does not necessarily indicate that cavefish can learn the numerosity of the objects in complete darkness. Actually, in this experiment, numerosity co-varied with non-numerical continuous quantities (volume, space, etc.) and the possibility remained that cavefish used these continuous cues instead of numbers. To demonstrate that animals use numbers, it is necessary to verify that they can discriminate different amounts of objects after the stimuli have been controlled for non-numerical variables. In Experiment 2, the same two subjects were tested in a more difficult task (0.5 ratio, two versus four objects); this time, half of the stimuli were controlled for continuous quantities and half were not controlled for continuous quantities (as in Experiment 1). Results showed that cavefish were able to discriminate between the two quantities only in the non-controlled condition in which both numerical information and continuous quantities were simultaneously available. When presented with stimuli controlled for continuous quantities, their performance dropped to the chance level. The result of this study aligns with previous literature on fish and other vertebrates. For instance, the performance of domestic cats, salamanders (*Plethodon cinereus*) and mosquitofish – initially trained to discriminate between sets of objects non-controlled for continuous quantities – was not significant as soon as stimuli controlled for continuous quantities

were presented (Agrillo et al., 2009; Krusche et al., 2010; Pisa and Agrillo, 2009).

Such results have often been interpreted through the prism of the 'last resort' theory (Davis and Perusse, 1988), according to which animals would preferentially use continuous quantities, as number processing would require a higher cognitive effort. However, it is worth noting that none of the abovementioned studies directly compared the performance in numerical and area discriminations. In the initial learning phase, subjects are usually provided with both numerical and continuous information, while in the probe trials only one of these two information types is available (number), making the task potentially more difficult for the absence of all previous cues. In line with this idea, Agrillo et al. (Agrillo et al., 2011) trained mosquitofish to discriminate between groups of geometric figures and found that the learning rate of trained mosquitofish did not differ when only numbers or only continuous quantities could be used to solve the task. In this sense, number processing does not seem to be more cognitively demanding than continuous quantity processing. The combination of number and continuous quantities represented the easiest condition, reinforcing the idea that the poor performance of mosquitofish with controlled stimuli reported in a previous study (Agrillo et al., 2009) was due to the lack of multiple cues instead of being the result of a true inability to process numerical information.

Similarly, two potential explanations could be advanced based on the results of Experiment 2: first, cavefish could have failed at discriminating the number-only condition because their neuro-cognitive systems might not be able to support number processing; second, their poor performance with controlled stimuli could be ascribed to the fact that fish were initially trained with non-controlled stimuli, only later were they presented with a combination of controlled and non-controlled stimuli. The possibility remained that this procedure might have affected the mechanisms adopted to discriminate between the quantities, leading the fish to use the combination of number and continuous quantities as reliable cues. To address this issue, we designed a follow-up experiment (Experiment 3) to assess whether cavefish can learn to discriminate between two and four objects when the two arrays were controlled for continuous quantities from the first stages of the training. All subjects proved able to select the reinforced numerosity, representing the first evidence of non-visual numerical abilities in fish. In this experiment, half of the subjects were trained toward the larger number as positive, and half of the subjects were trained toward the smaller one as positive; no difference in accuracy was found as a function of the type of reinforce, as previously reported in other teleost fish (Agrillo et al., 2009; Agrillo et al., 2010a; Agrillo et al., 2012a).

Fish tested in the visual domain also proved able to discriminate more difficult ratios [e.g. 0.67 ratio, two versus three objects (see Agrillo et al., 2009; Agrillo et al., 2011)]. In Experiment 4, we assessed whether the same cavefish tested in the previous experiment were also able to discriminate a 0.67 ratio solely using numerical information. No fish discriminated between two and three objects, providing clear evidence that the numerical acuity of trained cavefish does not exceed a 0.50 ratio, at least with the methodology we have described.

It would currently be challenging to understand why cavefish differ in numerical acuity from other fish species (Gómez-Laplaza and Gerlai, 2011b; Agrillo et al., 2009; Agrillo et al., 2010a; Agrillo et al., 2011). One may argue that the object representation using non-visual modalities might be less precise. In this sense, cavefish might share the same numerical systems reported in other teleost fish but exhibit a worse performance because of a general noise in

representing the items to be enumerated. However, we do not feel this is the case, as the same species proved able to learn shape discrimination between two 3D objects submerged in opposite positions of the tank, with apparently the same cognitive effort described in other species tested in the visual domain (Sguanci et al., 2010).

Another possibility is that pre-verbal numerical systems are more accurate in the visual modality. In regard to this topic, it is worth noting that there is a debate in human and non-human species as to whether (Tokita et al., 2013; vanMarle and Wynn, 2009) or not (Beran, 2012; Jordan et al., 2005; Jordan et al., 2008) numerical acuity is differently affected by the sensory modality involved. For instance, while some authors have suggested the existence in humans of a single supramodal and domain-independent numerical system (Izard et al., 2009; Barth et al., 2003) and have not observed any difference in numerical acuity between visual and non-visual modalities, a recent study (Tokita et al., 2013) reported a different performance in numerosity judgments tested in visual and auditory conditions, advancing the idea of multiple core number systems in which visual and auditory numerosities are mentally represented with different signal variabilities. This debate extends far beyond the scope of this paper. Nonetheless, the lower performance reported in the numerical acuity of cavefish is worth noting and is theoretically compatible with the idea of modality-dependent systems of number representation.

A completely different explanation is related to the peculiar niche to which *P. andruzzii* is adapted. This species evolved for millions of years in a homogeneous environment in the absence of natural predators and with a scarcity of food resources. Selective pressures might have acted differently from other fish species, on the one hand reducing the cerebral mass in order to optimize the metabolic consumption of the brain, and on the other hand losing the neural circuits supporting cognitive functions not useful in the cave, such as those necessary to discriminate the larger shoal (Buckingham et al., 2007; Gómez-Laplaza and Gerlai, 2011b; Hager and Helfman, 1991). *Phreatichthys andruzzii* shows an extreme troglomorphic phenotype, and the complete anophthalmia is accompanied by the complete absence of optic nerves and chiasm and by a strong reduction in the size of the entire brain (Berti et al., 2001; Ercolini and Berti, 1975). Furthermore, the schooling behavior is significantly reduced in the cave forms of *Astyanax mexicanus* relative to the surface form (Kowalko et al., 2013; Parzefall, 1983). The loss of this behavior could be adaptive in hypogean environments because the absence of predators and the scarcity of food resources render the clustering of the fish disadvantageous.

More investigation is necessary to unravel this question. Studies employing species that are active in both good and poor light conditions such as mormyrid fish (Schuster and Amtsfeld, 2002), and could thus be tested in the visual as well as in other sensory modalities, would be particularly important to increasing our understanding of the exact role of sensory information in quantity judgments.

MATERIALS AND METHODS

Subjects

Phreatichthys andruzzii is one of the best adapted species of subterranean fish. Scales and pigmentation are absent, oxygen consumption is reduced, and anophthalmia, accompanied by the loss of the optic nerve, is complete 1 month post-hatching (Berti et al., 2001; Berti and Messana, 2010). *Phreatichthys andruzzii* live solely in the phreatic layer of the Somalia desert, a geographic area that cannot be reached easily. This species is characterized by an extremely low recruitment rate. Furthermore, even

though they have high longevity (up to 30 years in captivity), *P. andruzzii* is a slow-growing species (Berti and Messana, 2010) with a reduced number of offspring.

As few individuals are available in captivity, we used the same experimental design commonly used to test numerical abilities in rare or endangered mammals and birds [e.g. orangutan, *Pongo abelli* (Vonk, 2014); gorilla, *Gorilla gorilla gorilla* (Vonk et al., 2014); macaque (Brannon and Terrace, 1998); bear, *Ursus americanus* (Vonk and Beran, 2012); elephant, *Loxodonta africana* (Perdue et al., 2012); dolphin, *Tursiops truncatus* (Jaakkola et al., 2005); and parrot, *Psittacus erithacus* (Pepperberg, 2006)]. A small number of individuals are used in each experiment and experimental hypotheses are tested by statistically analyzing the performance of each individual subject.

A total of six individuals were tested in this study: two subjects in Experiments 1 and 2, and four subjects in Experiments 3 and 4. All subjects were adult individuals, approximately 6 years old, of unknown sex.

Experiment 1: discrimination of two versus six objects

Apparatus and stimuli

Before starting the experiment, subjects were maintained in a 150 l one-stock aquaria containing natural gravel, an air filter and live plants. Both stock aquaria and experimental tanks were maintained at a constant temperature of $27 \pm 1^\circ\text{C}$ and kept in darkness as *P. andruzzii* possess deep brain photoreceptors and show strong negative phototaxis (Tarttelin et al., 2012). A dim red lamp was placed in the experimental room to allow the experimenters to conduct the tests. Light reaching the experimental tank was less than 2 lx. Before the experiment, fish were fed twice daily to satiation with Chironomidae larvae and commercial food flakes (Sera). During the experiment, subjects were fed only during training and test trials with commercial food flakes.

The experimental apparatus was composed of a circular tank (diameter: 40 cm, height: 26 cm) filled with 15 cm of water; the external walls of the tank were covered by black adhesive tape. A shelter (circular shape, diameter: 18 cm) was provided in the middle of the tank. To prevent the possibility that fish might develop a side preference (left/right) in the environment, both the experimental tank and the shelter were perfectly symmetrical and could not provide any geometrical information (Sovrano et al., 2003). A water pump that permitted water-filtering was placed within the shelter. The filter was positioned outside the experimental tank. An infrared video camera (Sony HDR-SR 11E) was suspended approximately 1 m above the tank and was used to record the position of the subjects during the test trials.

Stimuli were composed of groups of white plastic sticks (PVC, height: 26 cm). Their base could differ according to both shape (circular, rectangular, triangular) and size. (The area of the base ranged from 0.785 to 3 cm^2 .) Twenty different pairs of two and six objects were presented in a pseudo-random sequence. No control for continuous quantities was made in this experiment and therefore cues other than number (e.g. total volume, density or overall space occupied by the two arrays) could be used to discriminate between the two quantities.

Procedure

In order to compare numerical abilities of cavefish with those of other species tested in the visual modality, we used the same paradigm we have previously adopted to evaluate six different teleost fish (Agrillo et al., 2012b). Two different phases were set up: (1) the training phase and (2) the test phase. In the training phase, fish were gradually trained to feed in correspondence to the larger quantity of objects; in the test phase, we measured whether they had learned to discriminate between the two quantities.

Training phase

Two days before the beginning of the training, subjects were introduced in the experimental tank to familiarize them with the environment.

Step 1: Days 1–6, 12 overall trials

Two trials per day were administered: one in the morning and one in the afternoon. For each trial, stimuli (two groups composed of two and six sticks) were routinely inserted in two opposite places. The position of the

stimuli within the tank changed randomly across trials. Soon after stimuli were inserted, a food reward (the same food flakes previously used in the stock tanks) was provided among the sticks composing the larger group. Food was first immersed in a glass containing water and then dropped by a Pasteur pipette into the experimental tank. After the subject ate the reward, another food reward was inserted in the same place. In total, four food rewards were provided within each trial. Stimuli were then removed until the beginning of the next trial.

Step 2: Days 7–10, eight overall trials

The procedure was similar to that described in Step 1, with the exception that a food reward was provided regularly every 15 min (instead of waiting for the fish to eat each food reward).

Step 3: Days 11–13, six overall trials

The procedure was similar to that described in Step 2, with the exception that the position of the stimuli changed four times within each trial. Stimuli changed their position after each food reward.

Overall, stimuli were presented 26 times during the training phase.

Test phase

Days 14–24 (two trials each day, 20 total trials)

The procedure was partially similar to that described in the previous training. However, soon after having inserted the stimuli in opposite positions of the tank, during the test phase, the experimenter waited for 4 min before providing the food reward. A second reward was then provided after 4 min. After 5 min, stimuli were removed from the tank. All test trials were video recorded.

The subjects' choice was calculated from video recordings as the time spent by the subjects near each stimulus for the interval of 4 min elapsing between the introduction of the stimuli in the tank and the delivery of the first food reward, a period in which fish could not have used any cue to select the positive stimulus. In particular, we calculated the proportion of time spent near the reinforced quantity [(time spent near the reinforced quantity) / (time spent near the reinforced quantity + time spent near the non-reinforced quantity)]. Choice areas were defined by dividing the experimental tank into eight equal areas (see Fig. 1). As stimuli were hung on the walls, they were placed in proximity to the walls in two opposite choice areas.

Experiment 2: discrimination of two versus four objects

At the end of the test phase of Experiment 1, the same fish were tested with a new series of stimuli using the same apparatus and procedure of the test phase of Experiment 1.

In this experiment, fish were presented with a new numerical ratio of 0.50 (two versus four objects). Half of the stimuli were not controlled for non-numerical cues as in Experiment 1. As a consequence, both number and continuous quantities were simultaneously available. The other half was controlled for non-numerical cues by matching the total volume occupied by the stimuli in each set. Furthermore, given that density (inter-individual distance) is negatively correlated to the overall space encompassed by the most lateral objects, half of the sets was controlled for overall space (by matching the maximal distance between objects), whereas the second half was controlled for density (by matching the average distance between objects in the set). Cumulative surface area, volume, density and overall space represent the non-numerical variables most frequently controlled in numerical cognition studies (Kilian et al., 2003; Pisa and Agrillo, 2009; Gómez-Laplaza and Gerlai, 2013; Feigenson et al., 2002).

Fish were tested with 40 different pairs of stimuli, 20 controlled and 20 non-controlled. The two types of stimuli were presented in semi-random order.

Experiment 3: discrimination of two versus four objects using numerical information only

In this experiment, half of the subjects were trained toward the larger numerosity as positive; the other half of the subjects was trained toward the smaller numerosity as positive.

The experimental apparatus and procedure were identical to that described in Experiment 1 with the exception of the type of stimuli. Fish were

presented with a 0.50 ratio (two versus four objects) controlled for all continuous quantities that co-vary with numbers. In particular, during training, one-third of the stimuli were matched to 100% for the volume of the objects. In short, the overall volume of the objects included in the smaller group was equal to the overall volume of the objects presented in the larger group. However, a by-product of equalizing the volume was that smaller-than-average objects would be more frequent in the larger groups, and a subject could have used this cue instead of a number. To reduce this possibility, volume was controlled to 85% in another third of the stimuli, and, in the remaining one-third of the stimuli, it was controlled to 70%. As a consequence, in the 70% condition, the biggest object within each pair was shown in the larger set; in the 85% condition, the biggest object was presented in the larger set in half of the trials and in the smaller set in the other half of the trials. To assess whether there was any difference as a function of the type of control, in the first 12 trials of the test phase, fish were presented with each type of volume control (100, 85 and 70%; four trials each type). In the remaining eight trials, stimuli were matched to 100%. As such, should the fish discriminate between the matched quantities in the trials controlled to 100%, neither volume – matched to 100% – nor the individual object size – an unreliable cue in the training phase – could have played a key role. In addition, half of the stimuli were matched for the density and half were matched for space occupied. Twenty different pairs of stimuli were adopted to avoid the possibility that fish could have discriminated by applying pattern recognition of the arrays instead of using numerical information.

Experiment 4: discrimination of two versus three objects using numerical information only

At the end of the test phase, the four fish used in Experiment 3 were tested with a new series of stimuli using the same apparatus and the same procedure of the test phase of Experiment 3. In this experiment, fish were presented with a new numerical ratio of 0.67 (two versus three objects). Non-numerical variables were controlled in the same way as described for Experiment 3.

Statistical analysis

For each experiment, proportions were arcsine square root transformed (Sokal and Rohlf, 1995). Means \pm s.d. are presented. Statistical tests were carried out using SPSS 20.0.

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Competing interests

The authors declare no competing financial interests.

Author contributions

A.B. and C.A. designed the study; C.T. and C.A. collected the data; A.B., C.T. and C.B. analyzed and interpreted the results; and A.B., C.B., A.F. and C.A. wrote the paper.

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References

- Agrillo, C. and Miletto Petrazzini, M. E. (2013). Glimpse of ATOM in non-human species? *Front. Psychol.* **4**, 460.
- Agrillo, C., Dadda, M., Serena, G. and Bisazza, A. (2008). Do fish count? Spontaneous discrimination of quantity in female mosquitofish. *Anim. Cogn.* **11**, 495-503.
- Agrillo, C., Dadda, M., Serena, G. and Bisazza, A. (2009). Use of number by fish. *PLoS ONE* **4**, e4786.
- Agrillo, C., Piffer, L. and Bisazza, A. (2010a). Large number discrimination by mosquitofish. *PLoS ONE* **5**, e15232.
- Agrillo, C., Ranpura, A. and Butterworth, B. (2010b). Time and numerosity estimation are independent: behavioral evidence for two different systems using a conflict paradigm. *Cogn. Neurosci.* **1**, 96-101.
- Agrillo, C., Piffer, L. and Bisazza, A. (2011). Number versus continuous quantity in numerosity judgments by fish. *Cognition* **119**, 281-287.
- Agrillo, C., Piffer, L., Bisazza, A. and Butterworth, B. (2012a). Evidence for two numerical systems that are similar in humans and guppies. *PLoS ONE* **7**, e31923.
- Agrillo, C., Miletto Petrazzini, M. E., Tagliapietra, C. and Bisazza, A. (2012b). Inter-specific differences in numerical abilities among teleost fish. *Front. Psychol.* **3**, 483.
- Agrillo, C., Miletto Petrazzini, M. E., Piffer, L., Dadda, M. and Bisazza, A. (2012c). A new training procedure for studying discrimination learning in fish. *Behav. Brain Res.* **230**, 343-348.
- Agrillo, C., Piffer, L. and Adriano, A. (2013). Individual differences in non-symbolic numerical abilities predict mathematical achievements but contradict ATOM. *Behav. Brain Funct.* **9**, 26.
- Baker, J. M., Shivik, J. and Jordan, K. E. (2011). Tracking of food quantity by coyotes (*Canis latrans*). *Behav. Process.* **88**, 72-75.
- Barnard, A. M., Hughes, K. D., Gerhardt, R. R., Divincenti, L., Jr, Bovee, J. M. and Cantlon, J. F. (2013). Inherently analog quantity representations in olive baboons (*Papio anubis*). *Front. Psychol.* **4**, 253.
- Barth, H., Kanwisher, N. and Spelke, E. S. (2003). The construction of large number representations in adults. *Cognition* **86**, 201-221.
- Beran, M. J. (2008). The evolutionary and developmental foundations of mathematics. *PLoS Biol.* **6**, e19.
- Beran, M. J. (2012). Quantity judgments of auditory and visual stimuli by chimpanzees (*Pan troglodytes*). *J. Exp. Psychol. Anim. Behav. Process.* **38**, 23-29.
- Beran, M. J., Perdue, B. M., Parrish, A. E. and Evans, T. A. (2012). Do social conditions affect capuchin monkeys' (*Cebus apella*) choices in a quantity judgment task? *Front. Psychol.* **3**, 492.
- Berti, R. and Messana, G. (2010). Subterranean fishes of Africa. In *Biology of Subterranean Fishes* (ed. E. Trajano, M. E. Bichuette and B. G. Kapoor), pp. 363-367. Enfield, NH: Science Publishers.
- Berti, R., Durand, J. P., Becchi, S., Brizzi, R., Keller, N. and Ruffat, G. (2001). Eye degeneration in the blind cave-dwelling fish *Phreatichthys andruzzii*. *Can. J. Zool.* **79**, 1278-1285.
- Brannon, E. M. and Terrace, H. S. (1998). Ordering of the numerosities 1 to 9 by monkeys. *Science* **282**, 746-749.
- Buckingham, J. N., Wong, B. B. M. and Rosenthal, G. G. (2007). Shoaling decisions in female swordtails: how do fish gauge group size? *Behaviour* **144**, 1333-1346.
- Burt de Perera, T. (2004). Spatial parameters encoded in the spatial map of the blind Mexican cave fish, *Astyanax fasciatus*. *Anim. Behav.* **68**, 291-295.
- Cantlon, J. F. and Brannon, E. M. (2007). Basic math in monkeys and college students. *PLoS Biol.* **5**, e328.
- Carazo, P., Font, E., Forteza-Behrendt, E. and Desfilis, E. (2009). Quantity discrimination in *Tenebrio molitor*: evidence of numerosity discrimination in an invertebrate? *Anim. Cogn.* **12**, 463-470.
- Dadda, M., Piffer, L., Agrillo, C. and Bisazza, A. (2009). Spontaneous number representation in mosquitofish. *Cognition* **112**, 343-348.
- Davis, H. and Albert, M. (1986). Numerical discrimination by rats using sequential auditory stimuli. *Anim. Learn. Behav.* **14**, 57-59.
- Davis, H. and Perusse, R. (1988). Numerical competence in animals: definitional issues, current evidence, and a new research agenda. *Behav. Brain Sci.* **11**, 561-615.
- Davis, H., MacKenzie, K. A. and Morrison, S. (1989). Numerical discrimination by rats (*Rattus norvegicus*) using body and vibrissal touch. *J. Comp. Psychol.* **103**, 45-53.
- Dezfuli, B. S., Capuano, S., Magosso, S., Giari, L. and Berti, R. (2009). The lateral line system in larvae of the blind cyprinid cavefish, *Phreatichthys andruzzii*. *Anat. Rec.* **292**, 423-430.
- Ercollini, A. and Berti, R. (1975). Light sensitivity experiments and morphology studies of the blind phreatic fish *Phreatichthys andruzzii* Vinciguerra from Somalia. *Monit. Zool. Ital.* **6**, 29-43.
- Feigenson, L. (2007). The equality of quantity. *Trends Cogn. Sci.* **11**, 185-187.
- Feigenson, L. and Carey, S. (2005). On the limits of infants' quantification of small object arrays. *Cognition* **97**, 295-313.
- Feigenson, L., Carey, S. and Spelke, E. S. (2002). Infants' discrimination of number vs. continuous extent. *Cognit. Psychol.* **44**, 33-66.
- Garland, A., Low, J. and Burns, K. C. (2012). Large quantity discrimination by North Island robins (*Petroica longipes*). *Anim. Cogn.* **15**, 1129-1140.
- Gebuis, T. and Reynvoet, B. (2012a). The role of visual information in numerosity estimation. *PLoS ONE* **7**, e37426.
- Gebuis, T. and Reynvoet, B. (2012b). The interplay between nonsymbolic number and its continuous visual properties. *J. Exp. Psychol. Gen.* **141**, 642-648.
- Gómez-Laplaza, L. M. and Gerlai, R. (2011a). Can angelfish (*Pterophyllum scalare*) count? Discrimination between different shoal sizes follows Weber's law. *Anim. Cogn.* **14**, 1-9.
- Gómez-Laplaza, L. M. and Gerlai, R. (2011b). Spontaneous discrimination of small quantities: shoaling preferences in angelfish (*Pterophyllum scalare*). *Anim. Cogn.* **14**, 565-574.
- Gómez-Laplaza, L. M. and Gerlai, R. (2012). Activity counts: the effect of swimming activity on quantity discrimination in fish. *Front. Psychol.* **3**, 484.
- Gómez-Laplaza, L. M. and Gerlai, R. (2013). Quantification abilities in angelfish (*Pterophyllum scalare*): the influence of continuous variables. *Anim. Cogn.* **16**, 373-383.
- Hager, M. C. and Helfman, G. S. (1991). Safety in numbers: shoal size choice by minnows under predatory threat. *Behav. Ecol. Sociobiol.* **29**, 271-276.

- Hanus, D. and Call, J. (2007). Discrete quantity judgments in the great apes (*Pan paniscus*, *Pan troglodytes*, *Gorilla gorilla*, *Pongo pygmaeus*): the effect of presenting whole sets versus item-by-item. *J. Comp. Psychol.* **121**, 241-249.
- Hauser, M. D., Carey, S. and Hauser, L. B. (2000). Spontaneous number representation in semi-free-ranging rhesus monkeys. *Proc. R. Soc. Lond. B* **267**, 829-833.
- Hauser, M. D., Dehaene, S., Dehaene-Lambertz, G. and Patalano, A. L. (2002). Spontaneous number discrimination of multi-format auditory stimuli in cotton-top tamarins (*Saguinus oedipus*). *Cognition* **86**, B23-B32.
- Hunt, S., Low, J. and Burns, K. C. (2008). Adaptive numerical competency in a food-hoarding songbird. *Proc. R. Soc. Lond. B* **275**, 2373-2379.
- Izard, V., Sann, C., Spelke, E. S. and Streri, A. (2009). Newborn infants perceive abstract numbers. *Proc. Natl. Acad. Sci. USA* **106**, 10382-10385.
- Jaakkola, K., Fellner, W., Erb, L., Rodriguez, M. and Guarino, E. (2005). Understanding of the concept of numerically 'less' by bottlenose dolphins (*Tursiops truncatus*). *J. Comp. Psychol.* **119**, 296-303.
- Jordan, K. E., Brannon, E. M., Logothetis, N. K. and Ghazanfar, A. A. (2005). Monkeys match the number of voices they hear to the number of faces they see. *Curr. Biol.* **15**, 1034-1038.
- Jordan, K. E., Maclean, E. L. and Brannon, E. M. (2008). Monkeys match and tally quantities across senses. *Cognition* **108**, 617-625.
- Judge, P. G., Evans, T. A. and Vyas, D. K. (2005). Ordinal representation of numeric quantities by brown capuchin monkeys (*Cebus apella*). *J. Exp. Psychol. Anim. Behav. Process.* **31**, 79-94.
- Kilian, A., Yaman, S., von Fersen, L. and Güntürkün, O. (2003). A bottlenose dolphin discriminates visual stimuli differing in numerosity. *Anim. Learn. Behav.* **31**, 133-142.
- Kowalko, J. E., Rohner, N., Rompani, S. B., Peterson, B. K., Linden, T. A., Yoshizawa, M., Kay, E. H., Weber, J., Hoekstra, H. E., Jeffery, W. R. et al. (2013). Loss of schooling behavior in cavefish through sight-dependent and sight-independent mechanisms. *Curr. Biol.* **23**, 1874-1883.
- Krause, F., Bekkering, H. and Lindemann, O. (2013). A feeling for numbers: shared metric for symbolic and tactile numerosities. *Front. Psychol.* **4**, 7.
- Krusche, P., Uller, C. and Dicke, U. (2010). Quantity discrimination in salamanders. *J. Exp. Biol.* **213**, 1822-1828.
- Lipton, J. S. and Spelke, E. S. (2003). Origins of number sense. Large-number discrimination in human infants. *Psychol. Sci.* **14**, 396-401.
- Meck, W. H. and Church, R. M. (1983). A mode control model of counting and timing processes. *J. Exp. Psychol. Anim. Behav. Process.* **9**, 320-334.
- Merritt, D. J., Rugani, R. and Brannon, E. M. (2009). Empty sets as part of the numerical continuum: conceptual precursors to the zero concept in rhesus monkeys. *J. Exp. Psychol. Gen.* **138**, 258-269.
- Murofushi, K. (1997). Numerical matching behavior by a chimpanzee (*Pan troglodytes*): subitizing and analogue magnitude estimation. *Jpn. Psychol. Res.* **39**, 140-153.
- Pahl, M., Si, A. and Zhang, S. (2013). Numerical cognition in bees and other insects. *Front. Psychol.* **4**, 162.
- Panteleeva, S., Reznikova, Z. and Vygoniyailova, O. (2013). Quantity judgments in the context of risk/reward decision making in striped field mice: first 'count', then hunt. *Front. Psychol.* **4**, 53.
- Parzefall, J. (1983). Field observation in epigeal and cave populations of the Mexican characid *Astyanax mexicanus* (Pisces, Characidae). *Mémoires de Biospéologie* **10**, 171-176.
- Pepperberg, I. M. (2006). Grey parrot numerical competence: a review. *Anim. Cogn.* **9**, 377-391.
- Pepperberg, I. M. and Brezinsky, M. V. (1991). Acquisition of a relative class concept by an African gray parrot (*Psittacus erithacus*): discriminations based on relative size. *J. Comp. Psychol.* **105**, 286-294.
- Perdue, B. M., Talbot, C. F., Stone, A. M. and Beran, M. J. (2012). Putting the elephant back in the herd: elephant relative quantity judgments match those of other species. *Anim. Cogn.* **15**, 955-961.
- Piffer, L., Agrillo, C. and Hyde, D. C. (2012). Small and large number discrimination in guppies. *Anim. Cogn.* **15**, 215-221.
- Pisa, P. E. and Agrillo, C. (2009). Quantity discrimination in felines: a preliminary investigation of the domestic cat (*Felis silvestris catus*). *J. Ethol.* **27**, 289-293.
- Plaisier, M. A., Bergmann Tiest, W. M. and Kappers, A. M. L. (2009). One, two, three, many - subitizing in active touch. *Acta Psychol.* **131**, 163-170.
- Revkin, S. K., Piazza, M., Izard, V., Cohen, L. and Dehaene, S. (2008). Does subitizing reflect numerical estimation? *Psychol. Sci.* **19**, 607-614.
- Roberts, W. A. (2005). How do pigeons represent numbers? Studies of number scale bisection. *Behav. Process.* **69**, 33-43.
- Rugani, R., Fontanari, L., Simoni, E., Regolin, L. and Vallortigara, G. (2009). Arithmetic in newborn chicks. *Proc. R. Soc. Lond. B* **276**, 2451-2460.
- Rugani, R., Cavazzana, A., Vallortigara, G. and Regolin, L. (2013a). One, two, three, four, or is there something more? Numerical discrimination in day-old domestic chicks. *Anim. Cogn.* **16**, 557-564.
- Rugani, R., Vallortigara, G. and Regolin, L. (2013b). From small to large. Numerical discrimination by young domestic chicks. *J. Comp. Psych.* [Epub ahead of print] doi:10.1037/a0034513.
- Schuster, S. and Amtsfeld, S. (2002). Template-matching describes visual pattern-recognition tasks in the weakly electric fish *Gnathonemus petersii*. *J. Exp. Biol.* **205**, 549-557.
- Sguanci, S., Ceccolini, F. and Berti, R. (2010). Non visual discrimination of shapes in the blind cave cyprinid *Phreatichthys andruzzii* Vinciguerra 1924. *Ethol. Ecol. Evol.* **22**, 353-358.
- Smith, B. R., Piel, A. K. and Candland, D. K. (2003). Numerosity of a socially housed hamadryas baboon (*Papio hamadryas*) and a socially housed squirrel monkey (*Saimiri sciureus*). *J. Comp. Psychol.* **117**, 217-225.
- Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York, NY: WH Freeman & Co.
- Sovrano, V. A., Bisazza, A. and Vallortigara, G. (2003). Modularity as a fish (*Xenotoca eiseni*) views it: conjoining geometric and nongeometric information for spatial reorientation. *J. Exp. Psychol. Anim. Behav. Process.* **29**, 199-210.
- Tartelin, E. E., Frigato, E., Bellingham, J., Di Rosa, V., Berti, R., Foulkes, N. S., Lucas, R. J. and Bertolucci, C. (2012). Encephalic photoreception and phototactic response in the troglolobiont Somalian blind cavefish *Phreatichthys andruzzii*. *J. Exp. Biol.* **215**, 2898-2903.
- Teyke, T. (1989). Learning and remembering the environment in blind cave fish *Anoptichthys jordani*. *J. Comp. Physiol.* **164A**, 655-662.
- Tokita, M., Ashitani, Y. and Ishiguchi, A. (2013). Is approximate numerical judgment truly modality-independent? Visual, auditory, and cross-modal comparisons. *Atten. Percept. Psychophys.* **75**, 1852-1861.
- vanMarle, K. and Wynn, K. (2009). Infants' auditory enumeration: evidence for analog magnitudes in the small number range. *Cognition* **111**, 302-316.
- Vonk, J. (2014). Quantity matching by an orangutan (*Pongo abelii*). *Anim. Cogn.* **17**, 297-306.
- Vonk, J. and Beran, M. J. (2012). Bears 'count' too: quantity estimation and comparison in black bears, *Ursus americanus*. *Anim. Behav.* **84**, 231-238.
- Vonk, J., Torgerson-White, L., McGuire, M., Thueme, M., Thomas, J. and Beran, M. J. (2014). Quantity estimation and comparison in western lowland gorillas (*Gorilla gorilla gorilla*). *Anim. Cogn.* **17**, 755-765.
- Walsh, V. (2003). A theory of magnitude: common cortical metrics of time, space and quantity. *Trends Cogn. Sci.* **7**, 483-488.
- Ward, C. and Smuts, B. B. (2007). Quantity-based judgments in the domestic dog (*Canis lupus familiaris*). *Anim. Cogn.* **10**, 71-80.
- Xu, F. and Spelke, E. S. (2000). Large number discrimination in 6-month-old infants. *Cognition* **74**, B1-B11.
- Yoshizawa, M. and Jeffery, W. R. (2011). Evolutionary tuning of an adaptive behavior requires enhancement of the neuromast sensory system. *Commun. Integr. Biol.* **4**, 89-91.
- Yoshizawa, M., Gorički, S., Soares, D. and Jeffery, W. R. (2010). Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. *Curr. Biol.* **20**, 1631-1636.