

Numerical discrimination by frogs (*Bombina orientalis*)

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Received: 24 February 2014 / Revised: 7 July 2014 / Accepted: 30 July 2014 / Published online: 10 August 2014
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Abstract Evidence has been reported for quantity discrimination in mammals and birds and, to a lesser extent, fish and amphibians. For the latter species, however, whether quantity discrimination would reflect sensitivity to number or to the continuous physical variables that covary with number is unclear. Here we reported a series of experiments with frogs (*Bombina orientalis*) tested in free-choice experiments for their preferences for different amounts of preys (*Tenebrio molitor* larvae) with systematic controls for variables such as surface area, volume, weight, and movement. Frogs showed quantity discrimination in the range of both small (1 vs. 2, 2 vs. 3, but not 3 vs. 4) and large numerosness (3 vs. 6, 4 vs. 8, but not 4 vs. 6), with clear evidence of being able to discriminate numerosness even when continuous physical variables were controlled for in the case of small numerosness (i.e., 1 vs. 2), whereas in the case of large numerosness it remains unclear whether the number or surface areas were dominant. We suggested that task demands are likely to be responsible for the activation of different systems for small and large numerosness and for their relative susceptibility to quantitative stimulus variables.

Keywords Number cognition · Quantity discrimination · Analog magnitude system (AMS) · Object file system (OFS) · Frogs

Introduction

Some forms of numerical abilities, including cardinal and ordinal identifications (e.g., Brannon and Terrace 1998; Rugani et al. 2010a, b, 2011; Pepperberg and Carey 2012) and arithmetic calculations (e.g., Beran and Beran 2004; Baker et al. 2011; Pepperberg 2012; Rugani et al. 2009, 2011, 2013a; Ward and Smuts 2007), have been widely demonstrated in non-human animals.

Studies in human infants suggest that nonverbal numerical capacities are based on two separate systems: one that processes small numerosness: the Object File System (OFS); and the other that estimates larger numerical values: the analogue magnitude system (AMS, e.g., Feigenson et al. 2002; Coubart et al. 2014; Hyde and Spelke 2011; for a review see Feigenson et al. 2004; Hyde 2011). The OFS would be based on the capability of individuating each new object entering into a scene, to which a new file (“object file”) would be assigned and stored in the working memory (thus, numbers would be only implicitly represented in this system). The signature of the OFS is that there is a limit to the number (usually ≤ 3) of object files that can be simultaneously tracked and stored in the working memory (Trick and Pylyshyn 1994). Estimations involving larger numerosness (>3) would be dealt by the AMS. According to the Weber’s law, the functioning of that system would be ratio-dependent. As the ratio between the numbers to be discriminated becomes larger, response times decrease and accuracy increases (Gallistel and Gelman 1992).

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The existence of an OFS is debated in non-human animals. For instance, it has been not revealed in prosimian primates tested in conditions similar to those used on human infants (Jones and Brannon 2012). Moreover, evidence suggests that the AMS can deal also with discriminations involving small sets of numbers in a variety of different species (Cordes et al. 2001; De Hevia and Spelke 2009; Cordes and Brannon 2009; Cantlon and Brennon 2007; Brannon and Terrace 1998; Smith et al. 2003; Judge et al. 2005; Beran 2007; Pepperberg 2012; Rugani et al. 2013a, b; Stancher et al. 2013). Nonetheless, some evidence for an OFS has been reported. Rugani et al. (2008) trained young chicks (*Gallus gallus*) to peck at arrays of dots differing in their numerosity. Chicks successfully learned to discriminate 2 versus 3, but failed to learn to discriminate 4 versus 6, which suggests that the chicks were using the OFS rather than the AMS (because ratios were the same). Gross et al. (2009) showed a similar result with honeybees (*Apis mellifera*): Bees successfully learned to distinguish between 2 and 3, but not 4 and 6 items. Agrillo et al. (2007) reported a set-size limit (≤ 3) characteristic of the OFS in the numerical comparisons of mosquito fish, such that fish (*Gambusia hoolbrooki*) were more likely to move toward the larger of two shoals in comparisons of 1 versus 2, 2 versus 3, and 3 versus 4, but were not more likely to select the larger shoal for comparisons of 4 versus 5, 5 versus 6, 6 versus 7, or 7 versus 8. However, other reports in fish (*Xenotoca eiseni*) seem to suggest the use of the AMS even with small numerosity (Stancher et al. 2013). Chicks too appear to use the AMS also with small numbers in certain tasks (e.g., Rugani et al. 2010b, 2013a, b). New-born chicks were reared with two stimuli, each characterized by a different number of heterogeneous (for shape, color, and size) elements, and food was found in proximity of one of the two stimuli. At test, on day 3, chicks were presented with stimuli depicting novel elements (for shape, color, and size) representing either the numerosity associated or not associated with food. We found that chicks preferred the number associated with food in the 5 versus 10 and 10 versus 20 comparisons both when quantitative cues were unavailable (stimuli were of random sizes) and when the overall area or perimeter was equated (Rugani et al., 2013b). Using the same experimental paradigm, we also proved young chicks' capability to distinguish between two small (2 vs. 3), two large (6 vs. 9, 8 vs. 14, 4 vs. 6, and 4 vs. 8), and a small and a large number (2 vs. 8), even when the overall area and perimeter were equated. These data, and especially the discrimination between 2 and 8, demonstrated that small and large numbers can be processed via AMS (Rugani et al. 2014).

A most basic numerical competence is the ability to make judgments of size differences (e.g., “more than...,” “less than...”) between two or more sets (Davis and

Pérusse 1988). Such an ability would conceivably be at the basis of efficient foraging strategies. The theory of optimal foraging (Krebs 1974) states that animals, when faced with two or more food options, would choose the one that provides the greatest energetic gain. Proto-numerical discrimination is often found in ecological situations in which an animal has to choose between two sets made of identical food items and differing in their numerosity. Salamanders (*Plethodon cinereus*), when presented with two different quantities of live fruit flies (*Drosophila virilis*), choose the larger quantity in the comparisons 1 versus 2 and 2 versus 3. The salamanders were allowed to approach either alternative, and flies remained visible throughout the duration of the trial. Thus, salamanders were not required to memorize the two different quantities of food available, but instead were required to make a decision on the basis of what they were actually seeing (Uller et al. 2003). Robins (*Petroica longipes*) have been shown to be capable of selecting the larger of two quantities when they were presented with two sets of food items such as 6 versus 8, 8 versus 64, and 16 versus 64 (Gerland et al. 2012). Coyotes' (*Canis latrans*) could discriminate between quantities of discrete food items in the comparisons 2 versus 5, 1 versus 3, and 1 versus 4 (Baker et al. 2011). Domestic dogs (*Canis lupus familiaris*) select the larger amount of hotdog, in the comparisons 1 versus 4, 1 versus 3, 2 versus 4, 3 versus 5, 1 versus 2, 2 versus 3, 3 versus 4, 4 versus 5, and 5 versus 6 (Ward and Smuts 2007). Asian Elephants (*Elephas maximus*) when simultaneously presented with two baskets, each containing different numbers of fruit pieces, identified divergences of quantities up to 6 (i.e., 4 vs. 1, 3 vs. 1, 4 vs. 2, 5 vs. 3, 2 vs. 1, 3 vs. 2, 4 vs. 3, 5 vs. 4, and 6 vs. 5; Irie-Sugimoto et al. 2009). Orangutans (*Pongo pygmaeus*) compared two sets each containing a different number (within the range 1–6) of cereal bits and chose the larger quantities when these were presented simultaneously (Call 2000).

It should be noted, however, that whenever different numbers of identical items are used at test, changes in number correlate with changes in quantitative variables—also called “continuous physical variables” (e.g., volume, contour length, and surface area) that covary with numerosity. Unless continuous variables are controlled for, it is factually impossible to conclude that subjects' discriminations are based actually on numerical information.

Under which conditions animals would take into account numerosity versus continuous extent has been poorly considered in the comparative literature, as noted by Cantlon and Brennon (2007). One of the first attempts to control for the possible use of the quantitative information has been reported by Hauser et al. (2000). When observing an experimenter hiding some pieces of apple, one at a time into one opaque container and a different number of pieces of apple into another opaque container, monkeys (*Macaca*

mulatta) approached the container containing the larger quantity in the comparisons 1 versus 2, 2 versus 3, 3 versus 4, and 3 versus 5. To address the possibility that monkeys were attending to volume rather than number, in one control condition the authors compared 1 versus 3 pieces of apple equalizing the overall volume of the two sets. Monkeys again chose the larger number, showing that the numerical cues were independent of the quantitative ones (Hauser et al. 2000). However, other quantitative cues were not taken into consideration. Similarly, horses (*Equus caballus*) selected the greater of two quantities when comparing small numerosness: 1 versus 2 and 2 versus 3, even when the total surface area of the two sets was equalized (Uller and Lewis 2009). Again, contour length or total surface was not controlled for.

A few studies, though, experimentally controlled for possible use of quantitative cues, Cantlon and Brannon (2006) and Merritt et al. (2009) demonstrated that monkeys can order numerical values when all quantitative variables are irrelevant in the resolution of the task; Vallortigara et al. (2010a, b), Rugani et al. (2008, 2010b, 2014) showed that chicks (*Gallus gallus*) discriminate numbers when volume, area, and contour length are controlled for.

Among the so-called lower vertebrates, evidence is less clear. In salamanders (*Plethodon cinereus*), movement-related cues seem to be crucial for quantity discrimination of food prey (Krusche et al. 2010), whereas in fish, overall surface area appears to be crucial in discrimination of shoal numerosness (Gómez-Laplaza and Gerlai 2013). Indeed, whether fish (*Pterophyllum scalare*) would show any sensitivity to number per se when continuous physical variables are accurately controlled for is unclear (Gómez-Laplaza and Gerlai 2013).

Given the paucity of experiments that systematically controlled for the possible role of continuous physical variables on quantity discrimination in lower vertebrates, we investigated the ability of a frog species (*Bombina orientalis*) to choose the larger versus smaller number of food items. We devised a series of original experimental controls for the non-numerical variables in an attempt to disentangle the role of numerical versus quantitative cues. Moreover, we also tried to address the issue of the possible existence of a small number system (OFS) in these animals, separated from the system for the estimation of approximate magnitude (AMS).

The frogs *Bombina orientalis* belong to a primitive taxonomic group (suborder) characterized by the presence of several ancestral characters (called Archaeobatrachia, Familia: Bombinatoridae) and thus offer a unique chance to find archaic cognitive structures, allowing investigation of the origin of the proto-numerical abilities in vertebrates (Ren et al. 2009; Gissi et al. 2006).

In all the experiments, we exploited the predatory response that frogs manifest selectively toward moving prey (such as *Tenebrio molitor* larvae, used in the present study). The lack, in some anurans including *Bombina orientalis*, of frontal eyes and head movement produces a response to the stimuli which is ideal for scoring choice behavior. Such straightforward and clear-cut response consists in an abrupt movement of the frog toward the chosen prey, closely followed by a single tongue protrusion (Lettvin et al. 1968).

Experiment 1

The aim of the first experiment was to check whether frogs of the species *Bombina orientalis* are able to discriminate between small numerosness (≤ 3) by testing the comparisons 1 versus 2, 2 versus 3, and 1 versus 3, as it has already been shown to occur in mammalian (Ward and Smuts 2007; Irie-Sugimoto et al. 2009) and avian species (Rugani et al. 2008, 2013a; Pepperberg 2012).

Materials and methods

Subjects and rearing conditions

Seven adult frogs (*Bombina orientalis*) obtained from a commercial stock (Spagnoli & Casagrande Snc, Via Torre verde, 62, 38122 Trento) were used in experiments 1 and 2. Oriental fire-bellied toad (*Bombina orientalis*, Boulenger, 1890) is a semi-aquatic, small-sized frog (adult male and female are approximately 3–4 cm long) belonging to the Bombinatoridae family. The natural distribution of this species is in Korea, northeastern China, and Russia, but it is commonly bred and reproduced in captivity.

In the 3 years, before the experiments, frogs were group-housed in an aquarium (50 cm × 50 cm × 40 cm) with the ground completely covered of gray gravel. Water depth was of 10 cm, and water temperature was of $22 \pm 5^\circ$ C. An emerging island (15 cm × 10 cm), in the center of the aquarium, was used by frogs for the activities normally carried out on a dry ground (e.g., feeding). The aquarium was illuminated by one fluorescent lamp (15 W) with a photoperiod of 14-h light and 10-h dark.

Given that frogs respond only to moving prey (the movement itself seems to stimulate the predatory response) (Lettvin et al. 1968), they were fed every other day with live *Alphitobius diaperinus* and *Tenebrio molitor* mealworms. Due to the larva's small dimensions, each frog usually ate about 2–4 larvae in each meal; therefore, the amount of the food eaten varied slightly from meal to meal. In order to motivate the animals to the testing stimuli, 2 days before each testing session, frogs were food-deprived.

Materials and procedure

At the beginning of each testing session, frogs were individually transferred into the apparatus. This consisted of a rectangular plastic box (20 cm × 24 cm × 15 cm) lit from above (35 cm from the apparatus floor) by one 40-W fluorescent bulb. At the beginning of each trial, the subject was placed in the starting position, which was at the center of the apparatus, at about 10 cm from the closest wall (and facing it) and equidistant (8 cm) from the two lateral walls. Stimuli were positioned one to the right and one to the left with respect to the subject. Each stimulus was composed of a group of live *Tenebrio molitor* larvae immobilized by an entomological needle at about 0.5 cm from the ground, and each larva was arranged in the position shown in Fig. 1. The larvae were free to oscillate (mostly along the vertical plane, because of the larva's morphological structure) without touching each other. We made sure stimuli were moving during the test to elicit the frog's predatory response.

In order to make both groups of larvae simultaneously visible to the frogs, the larvae were aligned parallel to each other and at 45° with respect to the frog's sagittal axis, and the closest larva was 7 cm away from the frog in the starting position (Fig. 1). The minimum distance between the two groups of larvae was of about 6 cm. Moreover, within each group, the distance between the

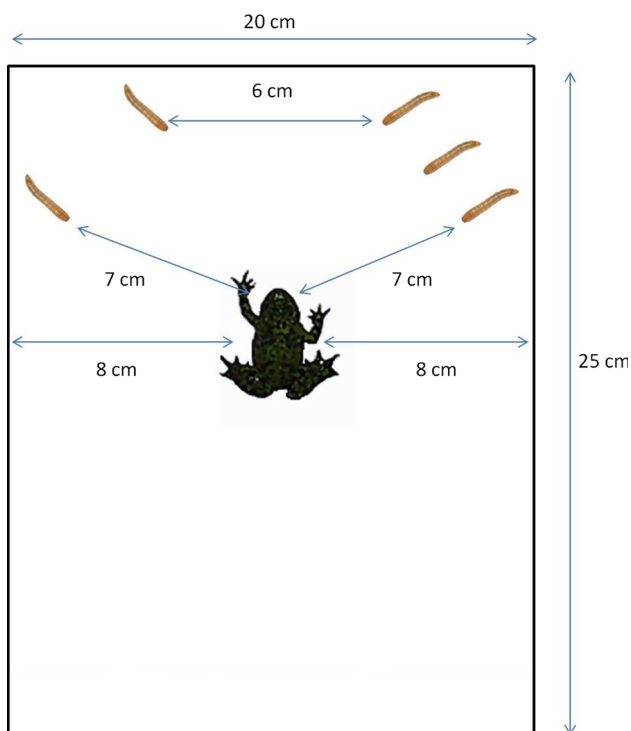


Fig. 1 A schematic illustration (*top view*) of the apparatus used in all of the experiments (a comparison 2 vs. 3 is shown)

larvae varied from a minimum of about 2 cm to a maximum of about 5 cm (depending on the numerosness of the group).

Each frog underwent three numerical comparisons: 1 versus 2, 2 versus 3, and 3 versus 4 (in this order). For each comparison, 15 valid testing trials were administered so that each subject underwent an overall of 45 valid trials. The delay between two consecutive trials was ≥ 60 min, and three trials were administered within each testing session, so that five testing sessions were necessary to complete each numerical comparison. One testing session took place every other 2 days. Larvae of the same size were employed in all testing trials for each comparison. The position (on the left -L- or on the right -R) of the larger group of larvae was changed from trial to trial, following a semi-random sequence (i.e., L-R-L-R-L-L-R-R-L-R-L-R-L-R-L, Fellows 1967) so that for each subject (and for each different comparison) the larger group was 46.667 % of times on the right side and 53.333 % on the left side.

In each testing trial, the subject was allowed a single choice. We considered the choice as a first attempt to catch any larva of any group. When the subject approached the larva at the distance of 1 cm, the choice was considered made, and the frog was placed back in the rearing aquarium. Only the first approach was scored. In no case, frogs were allowed to eat the larva, in order to exclude any possible learning effect. In this way, the subject's response was emitted in the context of a spontaneous choice task. If after 5 min the frog had not approached any stimulus, the response was considered non-valid and void and that trial was repeated (on average from 1 to 4 trials had to be repeated for each numerical comparison).

Results

An index of choice for the larger group was computed for each subject and for each comparison according to the formula: (number of choices of the larger set/15) × 100. An analysis of variance (ANOVA) was performed considering the three numerical comparisons (1 vs. 2, 2 vs. 3, 3 vs. 4) as repeated measures and, as dependent variable, the index of choice for the larger group. The ANOVA revealed a significant difference in frogs' responses to the different numerical comparisons ($F(2,12) = 3.888$; $p = 0.049$). A nonsignificant value in the Mauchly's sphericity test ($p = 0.240$) indicated variance homogeneity across groups. A Fisher's protected LSD post hoc analysis revealed a significant difference between the numerical comparisons 1 versus 2 and 3 versus 4 ($p = 0.017$).

One-sample *t* test (two-tailed) was used to estimate significant departures from random choice (50 %) in the overall sample of animals. The results showed a significant preference for the larger group in the comparisons 1 versus 2

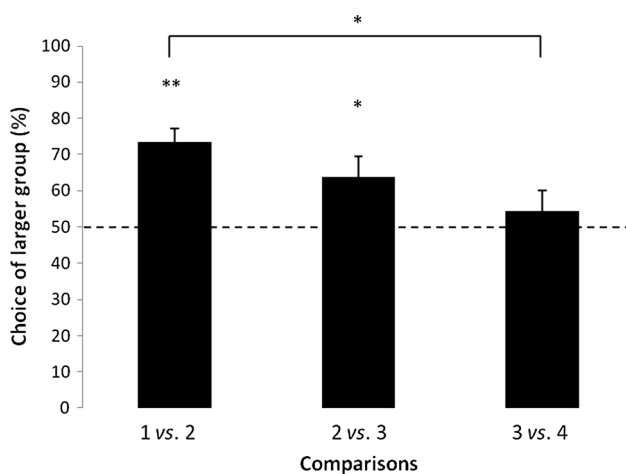


Fig. 2 The index of choice for the larger group (mean \pm SEM) for each numerical comparison of Exp. 1 (1 vs. 2, 2 vs. 3, and 3 vs. 4) is shown. The dotted line represents chance level (50 %). Asterisks indicate significant departures from chance level (* $p < 0.05$; ** $p < 0.01$)

($t(6) = 6.0619$; $p = 0.0009$) and 2 versus 3 ($t(6) = 2.4627$; $p = 0.0489$), but not in the comparison 3 versus 4 ($t(6) = 0.7499$; $p = 0.4816$) (see Fig. 2).

Experiment 2

In the previous experiment, we examined frogs' responses toward two numerically different groups of moving preys, obtaining a preference for the larger group in the case of the comparisons 1 versus 2 and 2 versus 3, but not in the comparison 3 versus 4. These data suggest a limit at about 3 items for the discrimination of these small quantities. However, the results can be explained by the functioning of the analogue magnitude system (AMS). The discrimination based on the AMS becomes less precise according to the Weber's fraction: As the ratio between the numerosness to be discriminated becomes larger, response times decrease and accuracy increases (Gallistel and Gelman 1992). The AMS should therefore support the discriminations when the ratio between the two numbers is small enough (as in the comparisons 1 vs. 2, ratio 0.5, and 2 vs. 3, ratio 0.67), but not when the ratio becomes closer to 1 (as in the comparison 3 vs. 4: ratio of 0.75). The second experiment aimed at extending the study to larger numerosness, using comparisons with a ratio of 0.5 (3 vs. 6, 4 vs. 8) or 0.67 (4 vs. 6).

Materials and procedure

Subjects, apparatus, and procedure were the same as described in the "Experiment 1" section. The only difference was the spatial arrangement of the stimuli, during



Fig. 3 Schematic representation of the apparatus and of the positioning of the stimuli in one of the numerical comparisons used in Exp. 2 (i.e., 4 vs. 8)

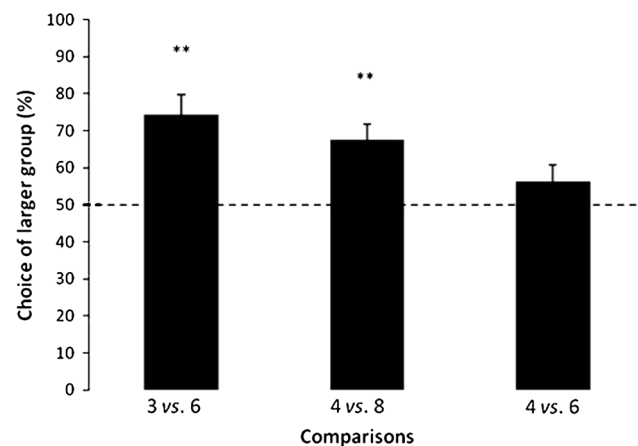


Fig. 4 The index of choice for the larger group (mean \pm SEM) displayed at test by frogs in the three numerical comparisons (3 vs. 6, 4 vs. 8, and 4 vs. 6) of Exp. 2. The dotted line represents chance level (50 %). Asterisks indicate significant departures from chance level (** $p < 0.01$)

each testing trial. Preys of the larger group were slightly misaligned, i.e., they were placed along two parallel lines (each 10 cm long) spaced about 6 mm apart (Fig. 3), while preys of the smaller group were placed along a single line (10 cm long). All frogs underwent three comparisons: 3 versus 6, 4 versus 8, and 4 versus 6.

Results

For each experimental comparison, the index of choice for the larger group was computed using the formula

previously described, see Fig. 4. A nonsignificant value in the Mauchly's sphericity test ($p = 0.432$) indicated variance homogeneity across groups. The analysis of variance revealed a nonsignificant difference between the three numerical comparisons ($F(2,12) = 3.324$; $p = 0.071$). Data were therefore merged, and the resulting mean (mean = 66.031; ES = 3.117) was above chance level: $t(20) = 5.143$; $p < 0.0001$.

Overall, the results of experiments 1 and 2 demonstrated that frogs can discriminate between small (1 vs. 2 and 2 vs. 3) and large (3 vs. 6 and 4 vs. 8) numerosness when a redundancy of cues was available. This may suggest that frogs do possess a system for evaluating the numerosness other than the OFS, a system that, in the realm of small numerosness, operates with a set-size limit of about 3 elements.

Nevertheless, in all these comparisons, the use of identical items (larvae of roughly same size) does not allow us to exclude the possibility that non-numerical cues were employed. Choice for the larger group could be indeed driven by quantitative cues. In the next experiments, a series of controls for some of the quantitative variables that may have affected the subjects' choice (volume, surface, and movement of the prey) is performed to check whether animals respond to the actual number instead of responding to the other quantitative features of the stimuli.

Experiment 3

The most relevant non-numerical cues that frogs may use in discriminating sets of food items are volume, surface, weight, and movement. The aim of the present experiment was to investigate the role played by the movement and volume in quantity discrimination of small numerosness (1 vs. 2). In catching behavior, movement is the most important cue to elicit a response toward a prey (Lettvin et al. 1968). On the other hand, volume also is a relevant cue typically used by frogs in the choice of food (Lettvin et al. 1968).

Subjects and procedures

A new group of 7 adult frogs were used. Rearing conditions, apparatus, and procedures were the same as in the previous experiments, unless otherwise noted.

Testing

Each subject underwent three separate tests employing the comparison 1 versus 2, each test controlled for the possible use of movement and/or volume.

First control (Moving and non-moving prey) To equalize the movement of the two stimuli, the one-element

stimulus was composed of a single moving mealworm and the two-element stimulus was composed of one moving mealworm and of one that could not move (as it was dead). The size of all of the mealworms was identical, and it was identical to that used in "Experiment 1" section.

Second control (Constrained movement) A control for the total amount of movement ("stuff in motion") was performed, using alive mealworms of the same size (identical to those used in Experiment 1). Using small "bobby pins," the one-element stimulus was immobilized in a single central position, so that both the anterior and the posterior parts of the body could oscillate, whereas in the two-element stimulus, both mealworms were fixed in two parts (center and posterior segments), so that they moved only the anterior portion of their body.

Third control (Partial occlusion of the prey) In this test, we controlled for both volume and movement at the same time. Six (three on each side) opaque, rectangular, and partially overlapping partitions ($3 \text{ cm} \times 2.5 \text{ cm}$) were introduced in the apparatus located at about 5 cm from the subject's starting point and spaced one from the other of 4 cm, see Fig. 5. These partitions could selectively hide some portions of the larvae body from the frog's visual perspective at the starting point. In order to obtain a control for both volume and movement, in the one-element stimulus, the live worm was entirely visible to the subject (no partition hid any part of the body of the larva). Conversely, for what concerns the two-element stimulus, only half body of each worm was visible (being each larva positioned in the space between two adjacent partitions): In particular, only the anterior half of a worm and only the posterior half

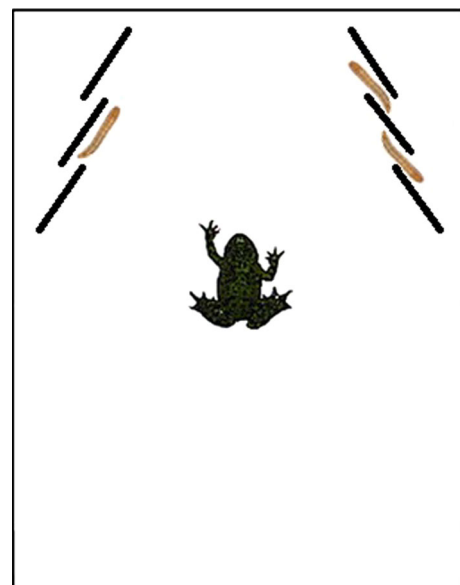


Fig. 5 Schematic representation of the apparatus used in Exp. 3 with the three partitions on each side and of the location of mealworms

of the other worm were visible to the frog in the starting point. In this way, possible preferences for attacking a certain side of the prey body were also controlled (usually the anterior side is preferred, as attacks are directed toward the head of a prey).

In order to obtain the best possible control for the quantity of movement in the two groups, in all these conditions, we made sure that all the living mealworms were moving during each trial. Whenever a mealworm was not moving, the trial was considered null and it was repeated immediately after.

Results and discussion

A within-subject ANOVA (Fig. 6) revealed a significant difference between the three control tests (First control “Moving and non-moving prey,” Second control “Constrained movement,” and Third control “Partial occlusion of the prey”), $F(2,12) = 5.576$; $p = 0.019$; a nonsignificant value in the Mauchly’s sphericity test ($p = 0.763$) indicated variance homogeneity across groups. A Fisher’s protected LSD post hoc analysis showed significant differences between the First control and the Second control ($p = 0.007$), and between the First control and the Third control ($p = 0.005$).

One-sample t tests (two-tailed) revealed a significant preference for the larger numerosness in the Second (“Constrained movement”: mean = 70.475; ES = 2.857; $t(6) = 7.166$; $p = 0.0004$) and Third (“Partial occlusion of the prey”: mean = 71.428; ES = 4.979; $t(6) = 4.303$; $p = 0.0051$) controls, but not in the First control (“Moving and non-moving prey”: mean = 52.380; ES = 4.467; $t(6) = 0.533$; $p = 0.6132$).

The results suggested that frogs were able to discriminate between small numbers of preys, at least in the

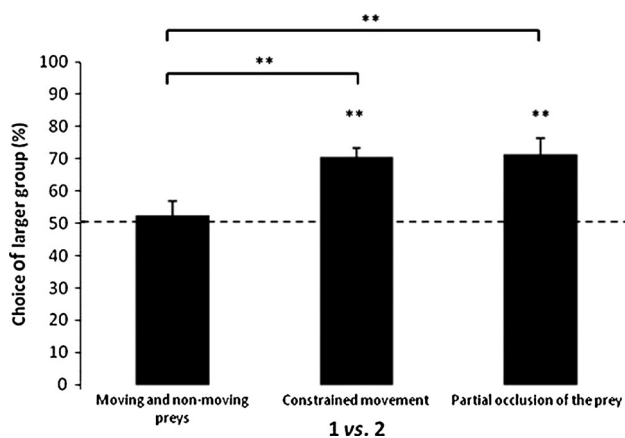


Fig. 6 Index of choice for the larger group (mean + SEM) displayed at test by frogs in each control of the Exp. 3. The dotted line represents chance level (50 %). Asterisks indicate significant departures from chance level (** $p < 0.01$)

comparison 1 versus 2, when the most relevant quantitative variables (movement and volume) were equalized. The lack of choice in the First control (Moving and non-moving prey) was probably due to the fact that the dead prey was neglected by the frogs, which usually much prefer to catch (at least partly) moving prey (Lettvin et al. 1968).

Experiment 4

Experiment 3 showed that frogs could discriminate between two groups of food items, when the total amount of volume and movement were controlled for. Nevertheless, in catching response, also the total weight of the preys could play a relevant role (Lettvin et al. 1968). In the present experiment, we used as stimuli live worms (*Tenebrio molitor*) of different sizes. The use of worms of different size allowed us to obtain two groups of the same weight that were tested in the comparisons 1 versus 2 and 4 versus 8.

Subjects and procedures

Subjects, rearing conditions, apparatus, and procedures were the same as in the previous experiment.

Testing

Frog underwent three tests, each controlling for the possible use of weight in discrimination of small or large numerosness.

Fourth control (1 vs. 2—Weight, volume, and surface control) We selected larvae of two sizes (“Large” larvae: 0.2 gr.; 32 mm × 4 mm, and “Small” larvae: 0.1 gr.; 21 mm × 2 mm) from two different molting stages, in order to equalize the overall weight of the two stimuli. Larvae were weighted using a Wunder Mix® high precision scale with a 0.01 gr. division. Moreover, we controlled for a possible preference for volume and surface, by creating a conflict between numerosness and quantity. To this purpose, the worm’s body was approximated to a cylinder; therefore, volume was calculated using the formula $V = \pi r^2 h$, lateral surface using the formula $LS = 2\pi r h$ (see Table 1). The one-element stimulus was composed of a larva having both the bigger volume and surface with respect to the sum of the volume and surface of the larvae composing the two-element stimulus. In this particular case, a choice for the larger group would indicate a preference for the larger number (i.e., 2), whereas the choice of the smaller group (i.e., 1) would indicate a choice of the bigger quantitative variables.

Fifth control (1 big vs. 1 small—Size control) To control for the possible preference of frogs for larvae of either size, we tested the comparison 1 versus 1 using larvae of the two

sizes (1 “small” and 1 “big”): The sizes of larvae were exactly the same of those used in the previous experiment (1 vs. 2—*Weight, volume, and surface control*).

Sixth control (4 vs. 8—Weight, volume, and surface control) The use of larvae of two different sizes (the same used in the “1 vs. 2—*Weight, volume, and surface control*”) made it possible a further control for the use of weight, surface, and volume in a comparison 4 versus 8 (4 “big” larvae vs. 8 “small” larvae). Again, the overall volume and surface were bigger in the group with less items, see Table 2.

Results

The index of choice for the larger (or bigger for the comparison 1 “small” vs. 1 “big”) group was calculated, for each experimental test (Fig. 7). Data were analyzed, separately for each testing session, employing a *t* test. A significant preference for the two-element stimulus was observed in the Fourth control (1 vs. 2—*Weight, volume, and surface*: mean = 60.952; ES = 3.390; $t(6) = 3.231$; $p = 0.017$). Frogs thus chose the numerically larger group, showing to prefer the numerosness over the volume and the surface.

A significant preference for the bigger worm was observed in the Fifth control (1 “big” vs. 1 “small”—*Size*: mean = 75.237; ES = 3.770; $t(6) = 6.695$; $p = 0.0005$). (In this case, the index of preference was calculated as number of choices for the “big” worm/number of total choices \times 100). The preference for the bigger worm thus confirmed that in the 1 versus 2—*Weight, volume, and surface control* test, the frogs’ choice for the two-element stimulus could be not due to a general preference for the smaller larvae.

No statistically significant preference was apparent for the Sixth control (4 vs. 8—*Weight, volume, and surface control*: mean = 42.856; ES = 5.607; $t(6) = 1.273$; $p = 0.249$).

Table 1 Weight, volume, and surface of the overall stimuli used in the 1 versus 2—*Weight, volume, and surface control* test (Exp. 4)

	1 “large”	2 “small”
Weight (gr)	0.2	0.2
Volume (mm ³)	401.9	131.8
Total surface area (mm ²)	401.9	263.6

Table 2 Weight, volume, and surface of the overall stimuli used in the 4 versus 8—*Weight, volume, and surface control* test (Exp. 4)

	4 “large”	8 “small”
Weight (gr)	0.8	0.8
Volume (mm ³)	1,607.6	527.2
Total surface area (mm ²)	1,607.6	1,054.4

Overall, these results indicated that both quantitative and numerical information could be processed by *Bombina orientalis* frogs in the discrimination of small sets of food items. Results from the “1 versus 2—*Weight, volume, and surface control*” test showed that the numerical cue was preferred over the quantitative cues (volume and surface). Interestingly, we did not notice any preference for the smaller larvae, because in the 1 “big” versus 1 “small”—*Size control* frogs showed a preference for the bigger larva. This also demonstrated that, when numerical information was not available, frogs could operate a discrimination on the basis of quantitative variables alone. Lack of difference in the 4 versus 8—*Weight, volume, and surface control* test suggests that large numerosness can be discriminated solely when numerical and non-numerical cues are both available (see “[Experiment 2](#)”).

Discussion

Although evidence for numerosness discrimination is widespread among non-human animals, both vertebrates (Vallortigara et al. 2010a, b) and invertebrates (Carazo et al. 2009; Pahl et al. 2013), accurate control for the role of continuous physical variables that covary with numerosness is relatively scanty, particularly in the so-called lower vertebrate species (for adequate controls in primates see however Brannon and Terrace 1998). In the present series of experiments, we systematically checked for the possible use of some quantitative cues (i.e., movement, volume, weight, and surface area) in a numerical discrimination task, and we showed that frogs are capable of discriminating small numerosness, even when the possible use of such quantitative physical variables is controlled.

The results of the present experiments provide some support to the hypothesis that frogs could respond to

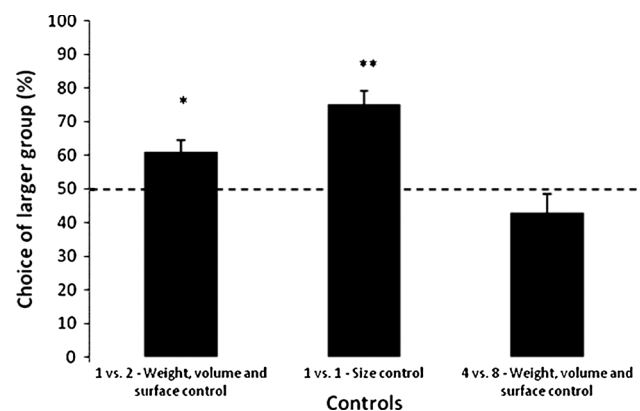


Fig. 7 Index of choice for the larger group (or for the larger larva in the “Size control” test) (means + SEM) displayed at test by frogs in each control of the Exp. 4. The dotted line represents chance level (50 %). Asterisks indicate significant departures from chance level (* $p < 0.05$; ** $p < 0.01$)

numerousness as such, at least with small numerousness. In the case of large numerousness (4 vs. 8), however, frogs appeared unable to discriminate in a condition in which the overall weight was equalized and when both volume and surface of the stimuli were larger in the group with less items (i.e., 4 large larvae vs. 8 small larvae). It is unclear whether this depends on the size of the numerousness or the test method used. With small numerousness, partial occlusion was used to equalize continuous physical variables, which proved, however, impossible to do (for spacing and distance reasons) using large numerousness. Given that in a comparison with only two larvae (1 vs. 1), frogs showed a preference for the larger insect, one possible explanation is that using 4 large larvae vs. 8 smaller ones the preference for the size of the single larva played against the preference for larger numerousness. It is true that the same could have occurred with the partly occluded larvae, but in that case, the “objective” difference in size between the visible parts could have been compensated by the non-visible ones through perceptual completion. Indeed, there is evidence in lower vertebrates for the perception of partly occluded objects as complete (e.g., Sovrano and Bisazza 2008).

These data suggest that frogs can discriminate stimuli on numerical basis exclusively via OFS, whereas the discrimination of larger numerousness, which is usually supported by the AMS, is possible solely when a redundancy of information, quantitative, and numerical is available.

An alternative account would be to imagine that there are two separate systems in frogs for small and large numerousness and that they are differently sensitive to continuous physical variables. Support to this hypothesis comes from the fact that frogs failed a discrimination with a ratio 2:3 in the range of large (>3) numerousness (discrimination 4 vs. 6), whereas they succeeded with small numerousness (discrimination 2 vs. 3, see Fig. 1). This would agree with the idea of a distinct small number system based on working memory and thus with an upper set limit of about 3 items (note that frogs failed the discrimination 3 vs. 4). In accord with this interpretation is also the fact that frogs did not show a significant difference in performance between the two successful discriminations 1 versus 2 and 2 versus 3, which is expected if these small numerousness discriminations would be treated by a separate object file system but not if treated by an approximate magnitude system because of their different ratios, 0.50 and 0.67, respectively).

This is not of course to argue that the small numerousness system would operate on numerousness independent of continuous physical variables, whereas the large numerousness system would not. This seems to be unlikely. Research on mammals and birds suggests that the AMS may operate on number. It is true, nonetheless, that most of

the neurons selective for numerousness in the monkey parietal cortex also show selectivity to continuous physical variables that covary with number, and only a minority respond only to number (Nieder and Dehaene 2009). It is tempting to speculate that this may be even more pronounced in lower vertebrates (or that perhaps lower vertebrates only have selectivity to quantitative variables but not to pure numerousness). In the absence of any hint about the neural machinery associated with quantity discrimination in these animals, this remains an open issue.

It is more likely, however, that the conditions that favor the use of either one of the two systems, and of their relative susceptibility to continuous physical variables, would depend on task demands. It has been suggested that small quantities may be represented by both the approximate magnitude (AMS) as well as the object file systems (OFS) and that contextual factors may determine which system is engaged (e.g., Wynn et al. 2002; Feigenson et al. 2004; Barner et al. 2008; Cordes and Brannon 2008, 2009; Hyde 2011; Rugani et al. 2013a, b). For instance, Hyde (2011) suggested that when items are presented under conditions that allow selection of individuals, they would be attended to by the OFS rather than the AMS, while when items are presented outside attentional limits (e.g., too many, too close together), they would be attended to by the AMS rather than the OFS. Prey catching in frogs clearly allows selection of individual elements as target of feeding responses, thus promoting use of OFS for small numerousness. When faced with large numerousness, however, tracking and targeting individual moving elements prove impossible, and use of surface area may represent the best strategy for selection of the group within which to perform targeting and prey catching. (Note that Wynn et al. (2002) and Barner et al. (2008) suggested that objects that undergo common motion are more likely to be represented as a collective entity than objects that move independently).

It is interesting to observe, in this regard, that discrimination between two shoals of conspecifics differing in the number of members in angelfish shows exactly the opposite pattern: Fish (*P. scalare*) used the number of shoal members as a cue only in large shoal contrasts but not in small shoal contrasts, where surface area dominated choice (Gómez-Laplaza and Gerlai 2013). Differently than prey catching, in which the attention is focused on a single individual, shoaling does not allow individuation of each element. Indeed, in the case of shoaling, the most relevant aspect is group size and not the identity of the individual members. Thus, comparison of numerical abilities associated with natural-occurring behavior in simple animals such as amphibians and other lower vertebrates may provide crucial information about the characteristics of the underlying mechanisms.

Acknowledgments This study was supported by the University of Padova (“Progetto Giovani” 2010, to R.R., prot.: GRIC101142 and “Progetto di Ateneo” 2012 to R.L. prot. CPDA127200) and by ERC Advanced Grant to G.V. (PREMESOR ERC-2011-ADG_20110406).

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