Quantity discrimination by treefrogs

Tyrone Lucon-Xiccato*, Elia Gatto & Angelo Bisazza

Dipartimento di Psicologia Generale, Università di Padova, Padova, Italy

*Correspondence: T. Lucon-Xiccato, Dipartimento di Psicologia Generale, Via Venezia 8, 35131, Padova, Italy. Phone: +39 0498277424; fax: +39 0498276600; e-mail: tyrone.luconxiccato@unipd.it

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Abstract

To make foraging, reproductive, and antipredator decisions, animals often have to discriminate discrete and continuous quantities (numbers and sizes of objects, respectively). Few studies have investigated discrete quantity discrimination in amphibians, but this has been done only in the context of prey selection. Using a species with arboreal habits, the Italian treefrog (*Hyla intermedia*), we investigated whether amphibians discriminate both discrete and continuous quantities when choosing between microhabitats. In field experiments, we showed that newly-metamorphosed treefrogs exhibit a preference for microhabitats with abundant and tall grass. In the laboratory, treefrogs presented with the dichotomous choice between two sets comprising different numbers of vertical green bars (simulating grass clumps) showed a preference for the larger set, and discriminated between 1 and 2 bars and between 2 and 4 bars, but not between 2 and 3 bars and between 3 and 4 bars. When presented with two bars of different size (i.e., one bar was taller and wider), treefrogs preferred the larger bar up to a 0.25 surface area ratio. Control experiments suggested that treefrogs represent numbers rather than continuous variables to discriminate between sets of bars and that they use the height but not the width of the bars to discriminate sizes. We also found evidence of a possible trade-off between speed and accuracy: individuals that chose more quickly did not display a significant preference for the larger bar/set of bars. These findings suggest that for amphibians, as for other vertebrates, a variety of decision-making processes can rely on quantitative abilities.

Keywords: *Hyla intermedia*; numerical abilities; quantity discrimination; speed-accuracy trade-off.

Introduction

Accumulating evidence suggests that animals discriminate quantities to make decisions in several contexts, such as when foraging, choosing social groups or reproductive partners (reviewed in Agrillo & Bisazza, 2014). The type of quantity to be discriminated may vary according to the context, and may be either discrete (i.e., the number of objects in a group) or continuous (i.e., the size of an object). For example, mammals, birds and fish, and even invertebrates, can discriminate the number of food items in a patch or the size of the individual food items in order to choose the larger food quantity (Bogale et al., 2014; Cross & Jackson, 2017; Lucon-Xiccato et al., 2015). Females of many species choose among potential mates based on the area of body colouration, or on the length of ornaments (Basolo, 1990; Griggio et al., 2011). Social fish choose from among available shoals based on the number and the size of the individuals in each shoal (Gómez-Laplaza & Gerlai, 2011; Ranta et al., 1992).

Research on vertebrates has revealed performance similarities across species and across tasks; this has suggested the existence of a cross-modal core system for processing quantity information inherited from a common ancestor (Beran, 2008; Feigenson et al., 2004). However, most studies have focused on only mammals, birds and fish, and on only one or just a few contexts in each species; thus, more comparative research is required to evaluate the aforementioned hypothesis. Only three studies have directly investigated quantitative abilities of amphibians, and the findings from these studies provide evidence of amphibians discriminating discrete quantities in the context of choosing their prey. Salamanders of the genus *Plethodon* can choose the larger group of live prey (fruit flies and crickets) well enough to discriminate 2 from 3 (Krusche et al., 2010; Uller et al., 2003). Similarly, oriental fire-bellied toads, *Bombina orientalis*, recognise and feed on the larger of two groups of 2 and 3 prey (Stancher et al., 2015). There is evidence that amphibians also use quantitative information in contexts other than prey selection (e.g., female mate choice: Arntzen, 1999; male calling: Gerhart et al., 2000), but these studies usually do not directly address the cognitive abilities and the mechanisms involved in the discrimination.

The main goal of the present study was to investigate amphibians' quantity discrimination ability in a context other than prey choice. For this, we focused on the microhabitat choices made by an arboreal anuran species, the Italian treefrog, *Hyla intermedia*. Arboreal anurans, especially the juveniles, climb vegetation upwards to evade terrestrial predators and/or to improve foraging (Stewart, 1985). It appears likely that microhabitats with more plants and larger plants will confer greater protection and contain more resources; thus, if vegetation is distributed in clumps or varies in size, treefrogs will display a preference for microhabitats with more plants or those with larger plants, as it has been observed in fish and lizards (Bartholomew, 2012; Cooper & Whiting, 2000). In agreement with this prediction, treefrogs in nature are more common in habitats with abundant vegetation (Ildos & Ancona, 1994) and they actively prefer microhabitats with vegetation (Micheals et al., 2014), which suggests that the observed distribution might be at least in part due to the treefrogs' behavioural preferences.

The treefrogs' microhabitat preference might be useful to study quantity discrimination, as it is commonly done with spontaneous preferences in other species (reviewed in Agrillo & Bisazza, 2014). We addressed this possibility by performing four experiments in the field (experiment 1, 2, 3, and 4). In experiment 1, we determined whether newly-metamorphosed Italian treefrogs living in grassland are attracted to microhabitats with abundant and tall grass, and whether they tend to climb such grass. In experiment 2, we tested whether treefrogs prefer larger grass clumps (both in leaves' number and size) using a dichotomous choice arena. These experiments showed that treefrogs exhibit a spontaneous preference for the larger available grass clump. To study quantity discrimination using such preference, it is important to control quantitative features of the stimuli, such as the area and height, and also features of the stimuli other than quantity, such as the colour. Since this is difficult using grass stimuli, we evaluated the use of artificial stimuli: in experiment 3 and 4, we tested whether treefrogs are attracted by green bars printed on paper and whether this attraction was similar to that for real grass, respectively.

The following four experiments addressed our main objective of investigating quantity discrimination during microhabitat choice. These experiments were performed in the laboratory to ensure controlled conditions (e.g., temperature, stimuli illumination) and thus reduce variability in subjects' behaviour. In experiment 5, we tested whether treefrogs could discriminate between different numbers of same-sized printed green bars and we identified the upper limit of their capacity for discrimination by presenting choices of 1 versus 4 bars, 2 versus 4 bars, 2 versus 3 bars, and 3 versus 4 bars. In experiment 6, we tested whether treefrogs could discriminate between two green bars of different sizes.

In experiments 7 and 8, we identified which object attributes are used by treefrogs to discriminate quantities. The discrimination ability determined in experiment 5 might have been brought about by the treefrogs representing the number of objects in a scene or, alternatively, it might been brought about by the treefrogs representing a value of a continuous variable that co-varies with numerosity, such the overall surface area or the convex hull (Davis & Pérusse, 1988). For example, as the bars of experiment 5 had the same size, the set with more bars also had larger surface area (i.e., the larger stimulus is more green) and treefrogs might have based their choices solely on this continuous variable. Some studies have supported the 'last resort' hypothesis: that animals most often use continuous variables to discriminate between discrete quantities and that they use numerical information only when relying on the continuous variables is not available (e.g., Vos et al., 1988). However, other studies have suggested that animals readily make spontaneous decisions based on numerosity instead of on the co-varying continuous variables (Ferigno et al., 2017). To disentangle these possibilities, in experiment 7, we observed treefrogs choosing between 2 and 4 bars with the same overall surface area (experiment 7a) or convex hull (experiment 7b).

We considered whether treefrogs could use two different attributes of the stimuli (i.e., the height and the width of the bars) when discriminating the size of the bars. If one attribute is more relevant for treefrogs' choice (e.g., taller plants might confer better protection from terrestrial predators than wider plants), they might choose based on that attribute only. It is also possible that height and width of plants covary in nature and that treefrogs have been selected to rely on one of these attributes in order to minimize neuronal resources required for the choice (Todd, 2001). In experiment 8, we investigated whether treefrogs were influenced more by height (experiment 8a) or by width (experiment 8b) when choosing the larger bar.

The last goal of this study was to ascertain whether trade-offs between speed and accuracy are important to treefrogs' quantity discriminations. For various cognitive tasks, gathering accurate information and comparing the available options take a considerable amount of sampling time (Chittka et al., 2009). Both long sampling times and wrong choices may be costly and may reduce fitness. It has been proposed that animals deal with this problem by trading off choice time and choice accuracy (Chittka et al., 2009). We hypothesised that treefrogs would show such a trade-off between speed and accuracy when discriminating quantities, so in the laboratory experiments we measured treefrogs' choice time to investigate this hypothesis.

Materials and methods

Animal welfare note

We adhere to the ASAB/ABS Guidelines for the Use of Animals in Research. The experiments complied with current legislation in the country (Italy) where they were conducted (Decreto Legislativo 4 marzo 2014, n. 26) and were approved by Università di Padova Ethical Committee (protocol n. 388523). No invasive physical manipulation was performed on the treefrogs. The treefrogs were kept in the laboratory for less than one day and then released into their natural environment.

Subjects

We collected treefrogs 15 days after metamorphosis in north-east Italy, near to Padova (45° 32' 30'' N, 11° 53' 40'' E). The population used in the study reproduces in an artificial pond. Each year, we observe a large number of egg masses (> 30). Although the adults of this species are known to inhabit trees and bushes, the subjects of this study were collected while climbing grass close to the pond (Fig. 1a). We collected the frogs in the morning between 06:00 and 07:00 using a wet hand net, placing them in a plastic box with grass for transfer. We tested groups of approximately 25 frogs each day, randomly assigning each animal to the different experimental conditions. The frogs transferred to the laboratory were kept at 26° C and provided with a dish of water and spray of nebulised water to keep part of the grass moist. In the field experiments, we tested 44 frogs overall, divided in the different experiments as follow: 8 in experiment 1, 12 in experiment 2, 8 in experiment 3, and 16 in experiment 4. In the laboratory experiments, we tested 328 frogs overall, 96 in experiment 5, 48 in experiment 6, 48 in experiment 7, and 136 in experiment 8. Each frog was tested only once.

Apparatus and procedures: experiments in the field

We performed experiment 1 in the same grassland where we collected the subjects to test whether treefrogs are attracted by grass and tend climb it. We released the subjects individually into the middle of an area (Ø 100 cm) that lacked tall grass, but that was surrounded on one side by grass 25-35 cm in height. We constructed this area by cutting and removing the grass. We performed the experiment using 4 replicates of the setting. We recorded the time that the subject took to reach the tall grass, whether the subject climbed the grass, and the time to climb to a height of 15 cm.

We performed experiments 2, 3 and 4 in a building close to the field site so that fresh grass could be used as a stimulus. We conducted these experiments in a white plastic circular arena (Ø 80 cm, height 75 cm; Fig. 1a). A LED spot lamp (100 watt, 1000 lumen, 100° angle) placed 100 cm above the middle of the arena illuminated the apparatus; this lamp was the only light source because we kept the arena in a dark room. This setting ensured absence of shadows and homogeneous illumination of the stimuli. A PVC tube (8 cm long) was connected to a hole (Ø 2.5 cm) in the middle of the floor of the arena and served as starting point for the subjects. This tube was inclined at a 45° angle to the floor of the arena. Before each trial, the experimenter positioned the stimuli against the wall of the arena, facing the exit from the PVC tube. The stimuli were either glass clumps or green bars printed on white papers (see below). Then, the experimenter collected one, randomly selected frog in a plastic jar and transferred it inside the PVC tube for a 30-s habituation. To start the trial, the experimenter slowly injected water inside the PVC tube with a 60 cl syringe and a silicone tube connected to the bottom of the PVC tube. This caused the frog to emerge in the arena, in which it could move freely (Supplementary material 1). The trial ended when the frog reached the wall of the arena and touched it, jumped on it, or stayed 10 s within one body length from it. We allowed the frog 30 min to reach the wall of the arena. If the frog did not move within 15 min, we interrupted the trial.

In experiment 2, we compared the choice between one microhabitat with a large grass clump and one microhabitat with a small grass clump. The stimuli were clumps of freshly collected grass leaves (Fig. 1b), attached to sheets of A4-size paper using transparent taper and placed on the walls of the arena. The distance between the two clumps was 45 cm. We recorded whether frogs chose the large clump or the small clump of grass. Our operational definition of choice for one stimulus was that the frog touched the stimulus, or it stayed within one-body-length of the wall in correspondence of the stimulus. We used 4 replicates of the stimuli.

In experiment 3, we tested whether frogs are attracted by green printed bars simulating vegetation. The stimuli were 12 green bars, each 2 cm wide and 28 cm high, printed on A3 sheet of paper separated by 2 cm of empty (i.e., white) space. Treefrogs are reported to perceive colours (Gomez et al., 2010), but their exact sensitivity is unknown; thus, we tried to match as close as possible the colour of grass in the field site. We used white paper as background to improve visibility of the bars. This stimulus actually consisted in alternating green and white bars, with the two colours having same area overall. We measured whether each subject touched a green bar or a white bar first. We then recorded the time spent on the green bars and the time spent on the white bars while the subjects were climbing for 2 min or until they reached the tip of the bars. Because green and white bars occupied the same surface area, if the frogs moved randomly toward the stimuli, they would be expected to choose the same number of times the green and the white bars, and to spend an equal amount of time over the green and white bars while climbing.

In experiment 4, we compared frogs' preference for printed bars and real grass. The stimuli were a single green bar, 1.5 cm wide and 25 cm high, printed on an A4 sheet, and one grass leaf of the same size attached to an A4 sheet. We recorded whether frogs chose the bar or the grass leaf.

Apparatus and procedures: laboratory experiments

Experiment 5, 6, 7 and 8 aimed at assessing treefrogs quantity discrimination abilities; we performed them in the laboratory to ensure controlled conditions. The experimental apparatus and the procedures were similar to that of experiments 2, 3 and 4. The stimuli were green printed bars with different number and size according to the experiment (see below). We recorded the frog's choice of the 'larger' or 'smaller' quantity and also recorded the time it had taken to reach the stimulus after emerging from the PVC tube. We conducted the experiments between 14:00 and 20:00 hours because a preliminary study showed that frogs are more attracted by the stimuli in the afternoon. Overall, 44 frogs did not complete the trial within the predetermined time or did not touch the wall in correspondence of the stimuli and were replaced. Replacement was done because frogs that did not choose between the stimuli do not provide information about discrimination ability.

In experiment 5, the stimuli were two sets with a different number of bars to study discrete quantity discrimination. Each bar was 1 cm wide and 28 cm high. Numerosity ratios and number of bars were as follow: numerosity ratio 0.25: 1 versus 4 bars; numerosity ratio 0.5: 2 versus 4 bars; numerosity ratio 0.67: 2 versus 3 bars; numerosity ratio 0.75: 3 versus 4 bars; Fig. 1c). The bars within each set were separated by a gap of 3 cm. We tested 24 frogs for each numerical ratio.

In experiment 6, we presented two bars of different size to study continuous quantity discrimination. By proportionally altering both height and width, we obtained ratios between the surface area of the two bars that corresponded to the numerical ratios significantly discriminated by the frogs in experiment 5 (ratio 0.25: a 1 × 12 cm bar versus a 2 × 24 cm bar; ratio 0.5: a 1.5 × 16 cm bar versus a 2 × 24 cm bar; Fig. 1d). We used the same ratios of experiment 5 to compare frogs' accuracy between discrimination of discrete and continuous quantities (Lucon-Xiccato & Dadda, 2017; Lucon-Xiccato et al., 2015). We tested 24 frogs for each size ratio. From this experiment onwards, we first tested the two ratios significantly discriminated in experiment 5 in order to minimise the number of wild animals needed to complete the experiment (as required by law in our country). We thus only tested frogs with larger ratios if they significantly discriminated the two ratios first administered.

From the results of experiment 5 it is not possible to ascertain whether frogs discriminate the two sets of bars based on bar number or on the continuous variables covarying with bar number (Davis & Pérusse, 1988). To address this point, in experiment 7, we sequentially controlled for the two most important attributes of the bars' sets that covary with numerosity, the overall surface area (sum of the surface area of each individual bar) and the convex hull (distance between the two most external bars of a set; Davis & Pérusse, 1988). In experiment 7a (control for overall surface area), the stimuli consisted of one set of 2 bars 2 × 28 cm in size and one set of 4 bars 1 × 28 cm in size; bars within the same set were separated by a 3 cm gap (Fig. 1e). In experiment 7b (control for convex hull), the stimuli included one set of 2 bars 1 × 28 cm in size separated by a gap of 11 cm, and one set of 4 bars 1 × 28 cm separated by a gap of 3 cm (Fig. 1e). If frogs fail the discrimination in one of these two controls, we would conclude that they likely discriminate between the two sets of bars based on the continuous variable corrected for. Conversely, if frogs choose the set with more bars in both experiments 7a and 7b, this would indicate that they base their choice on the number of bars. We tested 24 frogs in experiment 7a and another 24 frogs in experiment 7b.

From the results of experiment 6 it is not possible to ascertain whether frogs based their choice on the height of the bars or on the width of the bars, because the larger bar was both taller and wider than the smaller bar. We addressed this point in experiment 8 following the strategy of experiment 7 (i.e., sequential control of the attributes). In experiment 8a, we initially presented two bars of different height, based on the quantity ratios discriminated by the frogs in experiment 5 (ratio 0.25: a 1 × 7 cm bar versus a 1 × 28 cm bar; ratio 0.5: a 1 × 14 cm bar versus a 1 × 28 cm bar; Fig. 1f). Since we found a significant discrimination for both ratios, we then tested frogs with the two more challenging ratios used in experiment 5 (ratio 0.67: a 1 × 14 cm bar versus a 1 × 21 cm bar; ratio 0.75: a 1 × 21 cm bar versus a 1 × 28 cm bar; Fig. 1f). In experiment 8b, we presented two bars of different width based on the quantity ratios discriminated by the frogs in experiment 5 (ratio 0.25: a 1 × 28 cm bar versus a 4 × 28 cm bar; ratio 0.5: a 2 × 28 cm bar versus a 4 × 28 cm bar; Fig. 1g). As an example, if frogs discriminate between different-sized bars in experiment 8a but not in experiment 8b, then we would conclude that they base their choice on bar height rather than on bar width. We tested 24 frogs for each ratio in experiment 8a and 20 frogs for each ratio in experiment 8b.

Statistical analysis

The statistical analysis was performed in R version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). The statistical tests were two-tailed and the significance threshold was $P = 0.05$ if not stated otherwise. To study the preference of treefrogs for a certain stimulus, we compared the observed number of subjects choosing such stimulus with the number expected by chance (50 %) using chi-squared tests. In experiment 3, we additionally tested the preference for the green bars by comparing the percentage of time spent over the green bars with chance (50 %) using one sample *t*-test. In the laboratory experiments, the analysis was initially drawn separately for the different quantity ratios (experiments 5 and 6) and control conditions (experiments 7 and 8). For the experiments in which frogs were presented with different quantity ratios (experiments 5, 6 and 8), we then performed a cumulative analysis on all the ratios using generalised linear models (GLMs) with binomial error distribution and logit link function. As dependent variable, we used the choice of each frog (larger or smaller). We initially fitted the model with intercept only, to test whether frogs chose the larger stimulus overall, independently from the ratio; then, we fitted ratio as factor to test for differences between the ratios. In the experiments in which frogs were observed in more than two ratios (experiment 5 and 8a), we performed Tukey post-hoc test if the factor ratio was significant; we also tested for significant linear trend. To study speed-accuracy trade-off, we analysed frogs' choice in all the laboratory experiments using a GLM as described before. We fitted Log(choice time) as the covariate and experiment as the fixed effect. The interaction was omitted in the final model because it was not significant (Engqvist, 2005).

Results

Experiment 1 – Attraction to microhabitats with grass in nature

All 8 frogs rapidly reached the tall grass (time to reach the grass: 130.38 ± 50.32 s, mean ± standard deviation). After reaching the grass, all 8 frogs rapidly climbed it up to a height of 15 cm (climbing time: 65.12 ± 48.10 s).

Experiment 2 – Preference for larger grass clumps

Ten out of 12 frogs chose a stimulus. The two remaining frogs did not select any stimulus; one did not move for 15 min, at which point we interrupted the trial, and the other touched the white wall of the arena. Of the 10 frogs that made a choice, 9 chose the larger grass clump, and 1 chose the smaller one. The number of frogs choosing the larger stimulus (90 %) was significantly greater than chance (chi-squared test: $\chi^2_1 = 6.400$, $P = 0.011$).

Experiment 3 – Attraction to green printed bars

All the frogs reached the stimulus paper. Seven out of 8 chose the green bars first (Table 1), a preference that was significantly greater than chance (chi-squared test: $\chi^2_1 = 4.500$, $P = 0.034$). Overall, the frogs spent significantly more time climbing the green bars than the white bars (89.71 ± 10.20 % time spent over the green bars; one-sample *t* test against random choice: $t_7 = 24.746$, $P < 0.0001$; Table 1). The only frog that initially chose a white bar spent 87 % of its climbing time on the green bars.

Experiment 4 – Preference for green bars versus grass

Two frogs did not move for 15 min and were removed from the sample; the remaining 14 reached one of the stimuli. Six frogs chose the printed bar, and 8 chose the grass leaf; this difference was not significant (chi-squared test: $\chi^2_1 = 0.286$, $P = 0.593$).

Experiment 5 – Discrete quantity discrimination

In discriminating between 1 and 4 bars, 22 out of 24 frogs chose the stimulus with the larger number of bars; and in discriminating between 2 and 4 bars, 19 out of 24 frogs chose the stimulus with the larger number of bars. For both of these two easier ratios, the number of frogs choosing the larger number of bars was higher than expected by chance (1 versus 4: $\chi^2_1 = 16.667$, $P < 0.0001$; 2 versus 4: $\chi^2_1 = 8.167$, $P = 0.004$; Fig. 2a).

In discriminating between 2 and 3 bars, 14 out of 24 frogs chose the stimulus with the larger number of bars; in discriminating between 3 and 4 bars, 13 out of 24 frogs chose the stimulus with the larger number of bars. For both these two higher ratios, the number of frogs choosing the larger number of bars did not differ significantly from chance (2 versus 3: $\chi^2_1 = 0.667$, $P = 0.414$; 3 versus 4: $\chi^2_1 = 0.167$, $P = 0.683$; Fig. 2a).

When considering all the numerical ratios, the GLM showed that the number of frogs choosing the stimulus with the larger number of bars (68 out of 96; 70.83 %) was significantly greater than chance (estimate = 0.887, SE = 0.225, $z = 3.952$, $P < 0.0001$). The linear trend was significant ($P = 0.003$), indicating that the number of frogs choosing the larger number of bars decreased with increasing ratio between numerosities. The GLM revealed a significant difference between the ratios ($\chi^2_3 = 11.861$, $P = 0.008$). Post-hoc test found that the difference was significant between the 1 versus 4 bars and the 3 versus 4 bars discrimination ($P = 0.040$), close to the threshold for significance between the 1 versus 4 bars and the 2 versus 3 bars discrimination ($P = 0.069$), and not significant between the other numerosity ratios (1 versus 4 bars and 2 versus 4 bars: $P = 0.628$; 2 versus 4 bars and 2 versus 3 bars: $P = 0.411$; 2 versus 4 bars and 3 versus 4 bars: $P = 0.267$; 2 versus 3 bars and 3 versus 4 bars: $P = 0.991$).

Experiment 6 – Size discrimination

The number of frogs that chose the larger bar was significantly above chance in the 0.25 ratio (18 out of 24, $\chi^2_1 = 6.000$, $P = 0.014$; Fig. 2b) but not in the 0.5 ratio (13/24, $\chi^2_1 = 0.167$, $P = 0.683$; Fig. 2b).

When considering both size ratios, the analysis with the GLM showed that the number frogs choosing the stimulus with the larger bar (31 out of 48; 64.58 %) was significantly greater than chance (estimate = 0.601, SE = 0.302, $z = 1.991$, $P = 0.047$). The GLM did not find a significant difference between the ratios ($\chi^2_1 = 2.303$, $P = 0.129$).

Experiment 7 – Attributes exploited in discrete quantity discrimination

In experiment 7a (stimuli controlled for overall surface area), 17 out of 24 frogs chose the stimulus with the larger number of bars; in experiment 7b (stimuli controlled for the convex hull of the bars), 20 out of 24 frogs chose the stimulus with the larger number of bars. In both these tests, the number of frogs that chose the stimulus with the larger numerosity was significantly greater than chance (overall surface area: $\chi^2_1 = 4.167$, $P = 0.041$; convex hull: $\chi^2_1 = 10.667$, $P = 0.001$; Fig. 3a), suggesting that frogs do not discriminate between the two sets of bars based on overall surface area or convex hull.

Experiment 8 – Attributes exploited in discriminating size

When the two bars differed in height (experiment 8a), the number of frogs that chose the larger bar was greater than chance for the 0.25 and 0.5 ratios (18/24, $\chi^2_1 = 6.000$, $P = 0.014$; 18/24, $\chi^2_1 = 0.800$, $P = 0.014$, respectively), but not for the 0.67 and 0.75 ratios (15/24, $\chi^2_1 = 1.500$, $P = 0.221$; 13/24, $\chi^2_1 = 0.167$, $P = 0.683$, respectively; Fig. 3b). Considering all height ratios, the analysis with the GLM showed that the number frogs choosing the taller bar (64 out of 96; 66.67 %) was significantly greater than chance (estimate = 0.693, SE = 0.217, $z = 3.202$, $P = 0.001$). The linear trend was close to the threshold for statistical significance ($P = 0.076$). The GLM did not find a significant difference between the ratios ($\chi^2_3 = 3.771$, $P = 0.287$).

When the two bars differed in width (experiment 8b), the number of frogs choosing the larger bar did not differ from chance (ratio 0.25: 13/20, $\chi^2_1 = 1.800$, $P = 0.180$; ratio 0.5: 12/20, $\chi^2_1 = 0.800$, $P = 0.371$; Fig. 3c); this suggests that frogs do not base their choice between different-sized bars on bar width. Similarly, the analysis on both width ratios with the GLM showed that the number frogs choosing the wider bar (25 out of 40; 62.50 %) was not significantly greater than chance (estimate = 0.511, SE = 0.327, $z = 1.564$, $P = 0.118$). The GLM did not find a significant difference between the ratios ($\chi^2_1 = 0.107$, $P = 0.744$).

Speed-accuracy trade-off

In the GLM model to study speed and accuracy, we found a negative relationship between the likelihood of choosing the larger stimulus and time taken to make the choice ($\chi^2_1 = 11.190$, $P < 0.001$; Fig. 4), suggesting a speed-accuracy trade-off. There was no significant effect of experiment ($\chi^2_5 = 6.318$, $P = 0.277$).

Discussion

Many animal species are capable of discriminating discrete and continuous quantities in different ecological contexts (e.g., foraging, mate choice, social interactions). We have limited knowledge on how and in which contexts amphibians use quantitative information. Our experiments revealed that treefrogs rely on quantitative abilities to choose microhabitats: they show a spontaneous preference for larger glass clumps and, by studying this behaviour in the laboratory using a dichotomous choice test, we showed that they can discriminate between numbers and heights of objects simulating vegetation.

In four experiments in the field, we investigated the possibility to study quantity discrimination during microhabitat choice by treefrogs. Experiment 1 indicates that treefrogs are attracted to microhabitats with abundant and tall grass and that they tend to climb vegetation, as previously reported for closely related species (Ildos & Ancona, 1994; Michaels et al., 2014; Stewart, 1985). When presented with a dichotomous choice between different-sized grass clumps (experiment 2), treefrogs showed a preference for the larger one. This choice behaviour is in line with that observed in reptile and fish species (Bartholomew, 2012; Cooper & Whiting, 2000) and might be used to study quantity discrimination, provided that the stimuli can be finely controlled. In experiment 3, we found that treefrogs are also attracted by green printed bars on a white background, and that they climb them as observed for real plants. The subjects' attraction to the printed stimulus bars appears to be similar to their response to real grass (experiment 4). The green bars might be perceived similar to the grass, or they might allow crypsis. More importantly for the purpose of this study, the bars are stimuli that can be easily controlled and used in a laboratory setting to study quantity discrimination. The main advantage of this approach is that it is based on a spontaneous behaviour; thus, the ability showed by subjects likely resembles that expressed by the species in the nature (Agrillo & Bisazza, 2014).

In the remaining experiments of this study, we focussed on quantity discrimination abilities and mechanisms. In experiment 5, treefrogs presented with two sets of same-sized vertical green bars chose the set with a larger numerosity, discriminating significantly up to the 2 versus 4 bars discrimination (0.5 numerical ratio). This discrete quantity discrimination could be achieved either by representing the number of bars or the continuous variables that covary with numerosity (Davis & Pérusse, 1988). In the two conditions of experiment 7, we separately controlled the stimuli for the two more important continuous variables that covary with numbers (overall surface area and convex hull). This did not prevent the treefrogs from identifying the set containing more bars suggesting the use of numerical information to discriminate between the available options. There is an ongoing debate about the importance of numerical information in discrete quantity discrimination: some studies suggest that animals spontaneously tend to use continuous variables, and that they use numerical information as a ‘last resort’, when prevented from using continuous variables (Vos et al., 1988). Other studies align with the present report in suggesting, instead, that animals spontaneously use numerical information (Ferigno et al., 2017). Regarding amphibians, salamanders’ choice of the larger group of live prey seems to be driven by quantity of movement (Krusche et al., 2010); toads seem to spontaneously use numbers to discriminate prey groups, at least when the number of prey per group does not exceed 4 (Stancher et al., 2015).

In experiment 5, we also found a clear ratio effect indicating that treefrogs were more likely to choose the larger numerosity when the ratio between the number of bars was smaller. This finding supports the existence of an approximate number system for discrimination of small numerosities with an accuracy set by Weber’s law (Cantlon & Brannon, 2007). When testing each numerosity ratio separately, the maximum accuracy exhibited by the treefrogs in discrete quantity discrimination (2 versus 4: 0.5 ratio) was lower than previously reported in other anurans when choosing prey (e.g., 2 versus 3: 0.67 ratio; Stancher et al., 2015). There are at least four possible explanations for this result. First, there might be intraspecific differences in cognitive abilities (Clayton & Krebs, 1994; Day et al., 1999) and different anurans species might have a different numerical acuity. Second, anurans might perform differently in different tasks (e.g., in choosing between microhabitats as opposed to prey) because the different tasks are of different ecological relevance. For example, a social fish species, *Poecilia reticulata*, shows higher discrimination abilities when choosing between shoals comprising different numbers of conspecifics (0.8 ratio) than when choosing between groups of food items (0.5 ratio; Lucon-Xiccato et al., 2015; Lucon-Xiccato et al., 2016; Lucon-Xiccato et al., 2017); this might be due to the fact that choosing the larger social group is one of main antipredator defences of social fish (Seghers, 1974). Third, it is possible that anurans' numerical accuracy improves with age due to experience or ontogenic maturation of the nervous system (Bisazza et al., 2010). Accordingly, the reduced performance of our treefrogs can be due to the fact that they were very young individuals. One last possibility is that anurans show different motivation in the different tasks. Treefrogs might not exhibit a preference between microhabitats with small differences in number of plants because the choice confers limited advantages. This does not exclude the possibility that treefrogs perceive the difference between stimuli. The issue of motivation is typical of procedures based on spontaneous choices; future studies should try to address it by using discrimination learning procedures (Agrillo & Bisazza, 2014).

Experiment 6 investigated discrimination of continuous quantities (i.e., surface areas). Treefrogs presented with two bars of different size (one bar was taller and wider than the other bar) showed a preference for the larger one if the size ratio was 0.25, but not if the ratio was larger, suggesting that they discriminated sizes less well than numbers. In experiment 8, the heights and the widths of the bars were compared separately to see if one attribute was more important than the other. The treefrogs were accurate in discriminating height up to a ratio of 0.5 (coinciding with the most accurate discrimination in experiment 5), but they failed to discriminate bars of different width even with an easier 0.25 ratio. These results suggest that treefrogs are able to compare and discriminate continuous quantities as well as discrete quantities, thought we cannot exclude that treefrogs are differentially motivated in choosing between different number of grass leaves and different-size leaves. Further, the results suggest that when choosing the larger between different-sized objects, as in experiment 6, treefrogs choose the taller object but do not attend to differences in width between the objects. Indirect evidence of continuous quantity discrimination in amphibians has been previously provided by mate choice experiments: for example, male Andrew’s toad, *Bufo andrewsi*, show mating preference for larger females (e.g., Arntzen, 1999; Liao & Lu, 2009).

Overall, our study shows that treefrogs prefer larger clumps of vegetation and taller plants, a preference similar to that observed in other species (Bartholomew, 2012; Cooper & Whiting, 2000; Mensforth & Bull, 2008; Takahashi & Nagayama, 2016). Our study aimed to investigate the cognitive system underlying quantity discrimination and we did not address the functional significance of the behaviour that we observed. One can speculate that it might have evolved to avoid predators (Babbitt & Tanner, 1997). For a species such as the treefrog, which relies on cryptic colouration to defend itself, being in a large clump of vegetation is likely to offer greater protection. Likewise, treefrogs are arguably better protected against terrestrial predators when they climb taller plants. On the other hand, the ability to choose the larger clumps of vegetation and the taller plants might have evolved because it is advantageous during ambush feeding (Walsh & Downie, 2005), and/or because it favours homeostasis (Seebacher & Alford, 2002). In line with this idea, there is evidence that the choice for microhabitats with rich vegetation increases growth in treefrogs (Michelas et al., 2014). Testing these hypotheses will require field experiments in an effort to assess fitness advantages of microhabitat selection.

The last goal of our study was to investigate the effects of decision speed on accuracy in quantity discrimination. Decision speed is an important, but scarcely considered factor in cognitive performance (Chittka et al., 2009). Making accurate decisions often demands lengthy sampling times, which can be associated with costs. In our experiments, we found that long latencies were associated with a greater preference for the larger bar/set of bars. Previous studies have often considered similar results as an evidence of a speed-accuracy trade-off (e.g., Change et al., 2016; Lucon-Xiccato & Bisazza, 2016). Although our study did not provide direct evidence that choice time reflects time required for cognitive processing, our finding might indeed be another example of this pervasive association. In the case of treefrogs, the cost of lengthy sampling time might be that individuals spend more time outside the cover and consequently are longer exposed to potential predators.

Speed-accuracy trade-offs have been reported in various decisional processes in animals (e.g., Chittka et al., 2003; Latty et al., 2011; Wang et al., 2015), but it is only in humans that there is clear evidence of this phenomenon in quantitative tasks (Moyer & Landauer, 1967). In a recent experiment on fish’s shoal size discrimination abilities, we found that guppies switched more frequently between two shoals of conspecifics with increasing ratio between shoals’ numerosity (Lucon-Xiccato et al., 2017). This finding can be interpreted as indirect evidence of a longer sampling time being needed to perform challenging quantity discriminations. Taken together, the experiment with guppies and the present study with treefrogs suggest that speed-accuracy trade-offs probably affect quantity-based decision-making in non-human animals, too. Future research should directly study this trade-off and try to understand its causes (Chang et al., 2016; Lucon-Xiccato & Bisazza, 2016) and its ecological consequences (Chittka et al., 2003).

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Table

Table 1. Results of experiment 3.

Subject	First choice	Time climbing (s)	Time on the green bars (s)
1	Green bars	120	120
2	Green bars	120	91
3	Green bars	120	96
4	Green bars	55	52
5	Green bars	120	120
6	White bars	71	62
7	Green bars	120	120
8	Green bars	65	52

Figure captions

Fig. 1

(a) View from above of the arena used in experiments 2-8 and example of treefrog climbing grass in the field; stimuli used in (b) experiment 2, (c) experiment 5, (d) experiment 6, (e) experiment 7, and (f, g) experiment 8.

Fig. 2

Percentage of treefrogs choosing: (a) the set with the larger number of bars in experiment 5; and (b) the larger-sized bar in experiment 6. Dashed line indicates the chance level and asterisks significant deviations from chance ($P < 0.05$).

Fig. 3

Percentage of treefrogs choosing: (a) the set with the larger number of bars in experiment 7, when the stimuli were corrected for overall surface area (left bar) and convex hull (right bar); and the larger bar in (b) experiment 8a and in (c) experiment 8b. Dashed line indicates chance level and asterisks significant deviations from chance ($P < 0.05$).

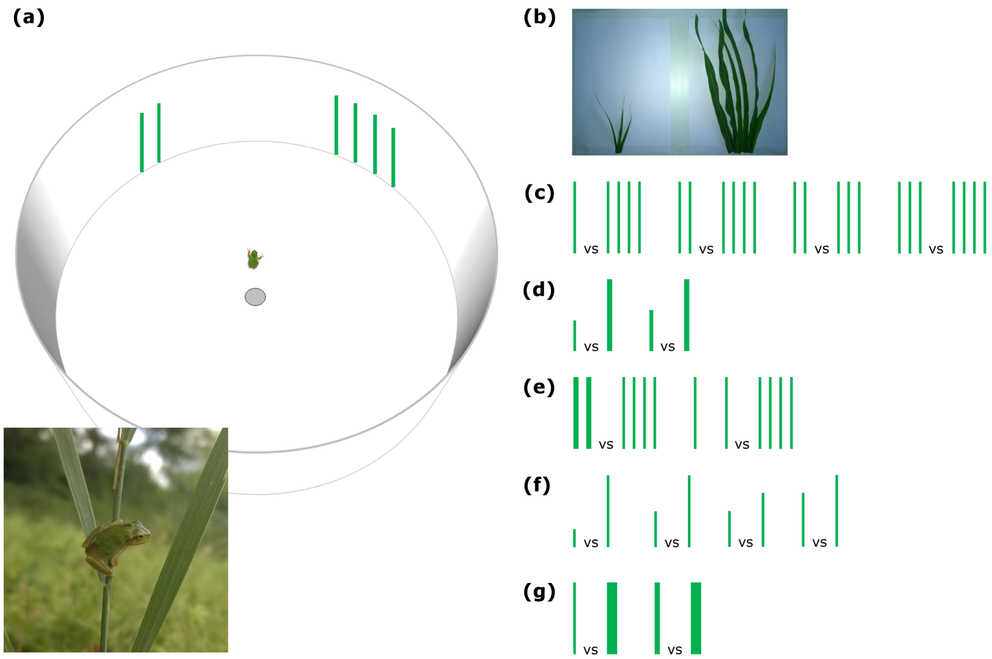
Fig. 4

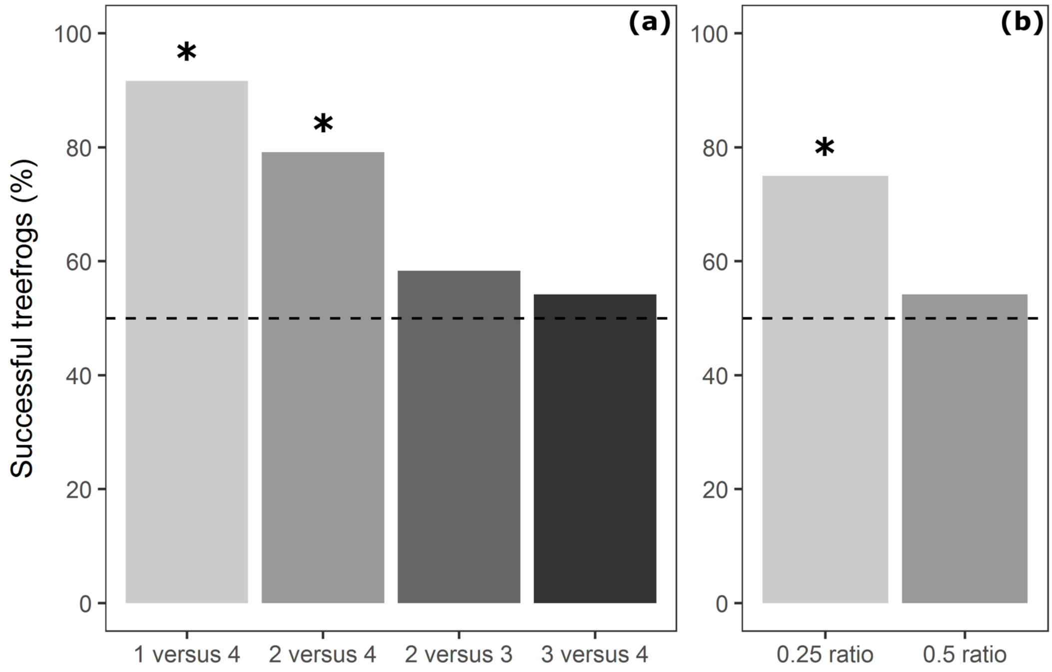
Accuracy in choosing the larger quantity as a function of time taken to make the choice. Points represent the choice made by the subjects (larger or smaller quantity) versus time taken to make the choice in experiments 5, 6, 7 and 8; the line and the grey shaded area represent speed accuracy relationship and CI predicted by generalised linear model with binomial error distribution and logit link function.

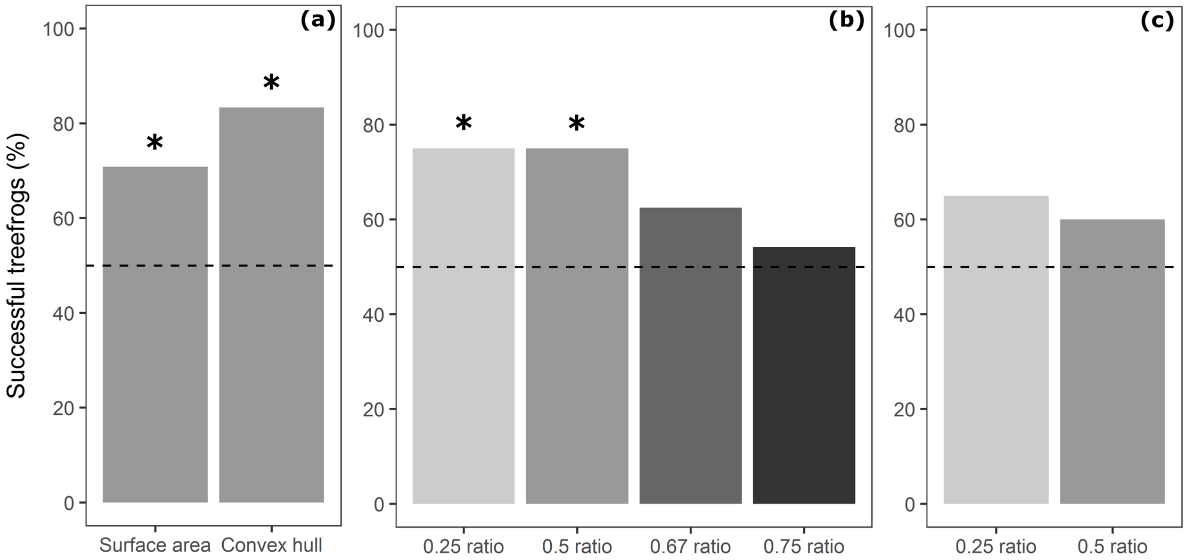
Supplementary material caption

Supplementary material 1

Video example of a trial. A treefrog choose between two sets with a different number of bars.







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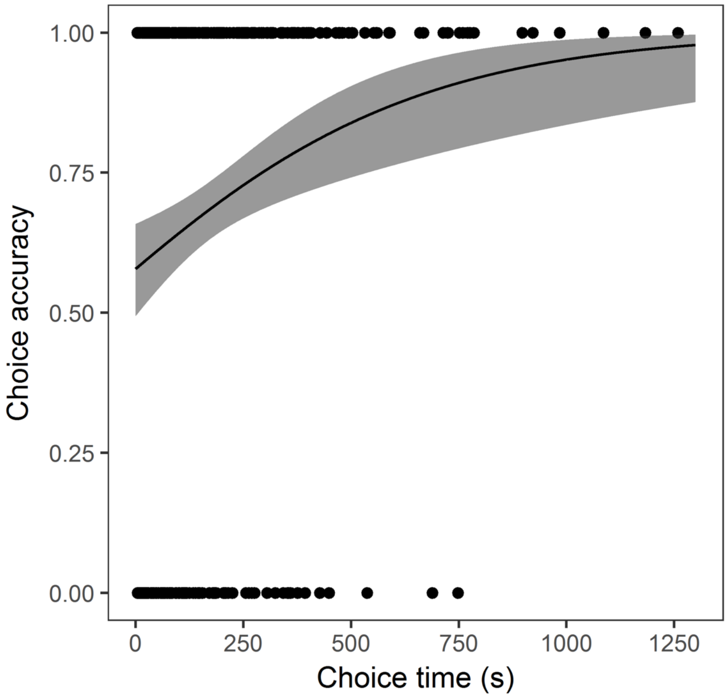

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