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Perception of the Müller–Lyer illusion in guppies

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Abstract

The Müller–Lyer illusion is a well-known distortion illusion that occurs when the spatial arrangement of inducers (i.e., inwards- or outwards-pointing arrowheads) influences a line's perceived relative length. To date, this illusion has been reported in several animal species but only in 1 teleost fish (i.e., redtail splitfins *Xenotoca eiseni*), although teleost fish represent approximately 50% of vertebrate diversity. We investigated the perception of this illusion in another teleost fish: guppies *Poecilia reticulata*, a species that diverged from the redtail splitfin 65 million years ago. The guppies were trained to select the longer between 2 lines; after meeting the learning criterion, illusory trials were presented. Control trials were also arranged to exclude the possibility that their choices were based on potential spatial biases that relate to the illusory pattern. The guppies' overall performance indicated that they were susceptible to the Müller–Lyer illusion, perceiving the line with the inwards-pointing arrowheads as longer. The performance in the control trials excluded the possibility that the subjects used the physical differences between the 2 figures as the discriminative cue in the illusory trials. Our study suggests that sensibility to the Müller–Lyer illusion could be widespread across teleost fish and reinforces the idea that the perceptual mechanisms underlying size estimation might be similar across vertebrates.

Key words: comparative perception, Müller–Lyer illusion, *Poecilia reticulata*, visual illusions

A visual illusion consists of the misperception of a visual cue due to the arrangement of various features, such as the surrounding context, the colors, or the light source's impact (Robinson 2013). For example, a target stimulus could be perceived as longer or shorter, larger or smaller, or brighter or darker depending upon the context in which it is presented. By looking at how a visual illusion is perceived, we can learn more about how the brain and perceptual mechanisms work. As a consequence, visual illusions have been used to investigate humans' visual interpretations. In the past few decades, this investigation has been extended to the animal world. The underlying evolutionary assumption is that if 2 or more species similarly perceive an illusory pattern, they are likely to share similar perceptual mechanisms (Feng et al. 2017; Parrish and Agrillo 2017). A robust investigation of animal sensibility to visual illusions could provide important clues about how evolution shapes visual perception.

Interestingly, animals could enjoy advantages in manipulating their relative dimensions or other physical features by exploiting illusory patterns (Kelley and Kelley 2013). For example, animals are often required to discriminate between sizes for fitness-related activities. When choosing partners, females often compare locally available males and choose among them depending upon characteristics that signal quality, including size (Andersson 1994). Camouflage colorations are another widespread example: disruptive colorations (i.e., the appearance of false edges and boundaries; Stevens and Merilaita 2008) make prey indistinguishable from the background and therefore difficult for predators to detect.

Given the large variety of ecological and anatomical differences in animal species, it is perhaps unsurprising that species differ in their perceptions of visual illusions. For example, chimpanzees (Parrish and Beran 2014) and bearded dragons (Santacà et al. 2019) demonstrated a human-like perception of the Delboeuf illusion (i.e., an object is perceived to be larger when surrounded by a smaller object), whereas dogs did not perceive this (Byosiere et al. 2017; Miletto Petrazzini et al. 2017). Also, dolphins were shown to experience the Ebbinghaus–Titchener illusion (Murayama et al. 2012), a similar illusion compared with the Delboeuf illusion, whereas baboons showed no susceptibility (Parron and Fagot 2007), and pigeons demonstrated

susceptibility to this visual illusion but in a reversed way compared with that of humans (Nakamura et al. 2008).

The Müller–Lyer illusion is one of the most investigated size illusions in the literature, and it occurs when inwards-pointing arrowheads (> <) and outwards-pointing arrowheads (< >) bind 2 identical spatial extends (i.e., lines; see Feng et al. [2017] for a review; Figure 1). Humans overestimate the line with "> <" ends, and they underestimate the one with "< >" ends. According to Gregory's inappropriate constancy-scaling theory (Gregory 1963), the outwards-pointing arrows are arranged in a configuration that makes the target line appear closer than the line with the inwards-pointing arrows, which make the line appear further away; this pattern would induce the relative size constancy scaling of the lines. Pressey (1972) suggested a different theory, namely the assimilation theory, according to which the longer overall size of the stimulus with inwards-pointing arrowheads "> <" makes the line appear longer than the line with outwards-pointing arrowheads "< >." Alternatively, Howe and Purves's (2005) competing probabilistic theory proposes that in the natural environment, "> <" ends are

more likely to indicate longer lines. Therefore, the Müller–Lyer bias could be due to a probabilistic strategy of visual processing.

The Müller–Lyer illusion has been investigated in different animal species (see Table 1). The tested mammals include capuchin monkeys *Cebus apella* (Suganuma et al. 2007), rhesus monkeys *Macaca mulatta* (Tudusciuc and Nieder 2010), and dogs *Canis familiaris* (Keep et al. 2018). Some bird species have been tested: for example, ringneck doves *Streptopelia risorii* (Warden and Baar 1929), homing pigeons *Columba livia* (Malott et al. 1967; Nakamura et al. 2006, 2009), and an African gray parrot *Psittacus erithacus* (Pepperberg et al. 2008). Regarding fish, only one cartilaginous fish, bamboo sharks *Chiloscyllium griseum* (Fuss et al. 2014), and one teleost fish, redtail splitfins *Xenotoca eiseni* (Sovrano et al. 2015), were investigated. Of all of these species, only bamboo sharks and dogs did not seem susceptible to the Müller–Lyer illusion (Fuss et al. 2014; Keep et al. 2018), whereas all of the others appeared to perceive the illusion as humans do (see Table 1 for information regarding the abovementioned studies). It is important to notice that teleost fish represent 50% of vertebrate biodiversity, and apart from Sovrano et al.'s (2015) study on redtail splitfins, we record the paucity of studies on the Müller–Lyer illusion among teleost fish. The Müller–Lyer bias might be relevant to fish in their natural environment for successfully navigating and catching food (e.g., for estimating distances and sizes). The human-like perception reported in redtail spitfins contrasts with the lack of illusory perception of bamboo sharks (Fuss et al. 2014), raising the intriguing possibility that teleost and cartilaginous fish display different perceptual mechanisms for size discrimination. Various factors could explain such a difference. For example, redtail splitfins are omnivorous fish that live in freshwater environments, and their diet consists largely of green matter, whereas bamboo sharks are carnivores that live in saltwater (Feng et al. 2017). Investigating other fish species would help us to shed light on whether ecological factors have a significant impact on shaping perceptual systems. All of the abovementioned investigations were conducted using a training procedure. In a pre-test phase, animals were trained to select the longer between 2 lines, and after having met the learning criterion, they could face the illusory pattern. A key aspect of the investigations of the Müller–Lyer illusion regards the fact that animals could select the line with "> <" ends, not
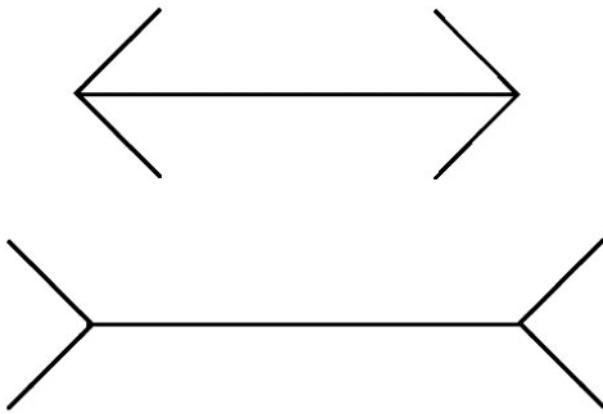
Figure 1. Müller–Lyer illusion. This illusion occurs when 2 same-length lines are perceived to be different depending upon whether arrowheads at each end point toward or away from each other.

Table 1. Information on the studies assessing non-human animal susceptibility to the Müller–Lyer illusion

Taxa	Species tested	Sample size	Susceptible?	Reference
Mammals	Capuchin monkeys *Cebus apella*	10	Yes	Suganuma et al. (2007)
	Rhesus monkeys *Macaca mulatta*	2	Yes	Tudusciuc and Nieder (2010)
	Dogs *Canis familiaris*	7	No	Keep et al. (2018)
Birds	Ringneck doves *Streptopelia risoria*	2	Yes	Warden and Baar (1929)
	Homing pigeons *Columba livia*	5	Yes	Malott et al. (1967)
	Homing pigeons *Columba livia*	3	Yes	Nakamura et al. (2006)
	Homing pigeons *Columba livia*	3	Yes	Nakamura et al. (2009)
	African gray parrot *Psittacus erithacus*	1	Yes	Pepperberg et al. (2008)
Fish	Bamboo sharks *Chiloscyllium griseum*	8	No	Fuss et al. (2014)
	Redtail splitfin fish *Xenotoca eiseni*	6	Yes	Sovrano et al. (2015)

because they perceive it as longer, but because they could simply select the longer overall figure. This leads to the same results as those that show a sensibility to the Müller–Lyer illusion. Although a human observer could easily be instructed to attend only to the lines and to ignore the arrowheads, instructing animals to do the same is unfeasible. Therefore, to exclude such a possibility, one must arrange specific control trials (Keep et al. 2018).

In the present study, we investigated susceptibility to the Müller–Lyer illusion in a teleost fish, the guppy *Poecilia reticulata* to understand how widespread the perception of this illusion is across vertebrates. To date, 4 visual illusions have been investigated in guppies: the brightness illusion (Agrillo et al. 2016), the Rotating Snakes illusion (Gori et al. 2014), the Delboeuf illusion (Lucon-Xiccato et al. 2019) and the Solitaire illusion (Miletto Petrazzini et al. 2018b). The guppies perceived all 4 illusions in a human-like way, with the exception of the Delboeuf illusion, where they experienced a reversed illusion (perceiving as larger what human observers commonly perceived as smaller). To investigate guppies' susceptibility to the Müller–Lyer illusion, we adopted a training procedure. Subjects were trained to choose the longer between 2 lines, regardless of the presence or position of the arrowheads, to get a food reward. Only the subjects that learned such a discriminative rule passed to the test phase, during which they faced the illusory pattern. Additional controls were included to exclude the possibility that the physical difference (namely, the overall figure length) between the 2 illusory figures influenced the subjects' performance in the illusory trials.

Materials and Methods

Experimental subjects

We preliminarily assessed the sample size using Fisher's exact test, choosing $\alpha = 0.05$ and a desired power of 0.80. Assuming a Cohen's d of 1.06 (data taken from Lucon-Xiccato et al. 2019), we found that a total of 10 subjects was appropriate for testing our hypothesis. We accordingly stopped our data collection until we recruited at least 10 guppies that completed the experiment.

The experimental subjects included 27 adult female guppies that belonged to the "snakeskin cobra green" strain, an ornamental strain that we regularly breed in our laboratory in the Department of General Psychology (University of Padova, Italy). Because some fish stopped participating (6) or did not meet the training phase's learning criteria (9), the final sample that we used for the statistical analyses comprised 12 trained guppies. The guppies were maintained in mixed-sex groups in 400-L tanks that were lined with gravel and enriched with natural vegetation (*Hygrophila corymbosa* and *Taxiphyllum barbieri*). A biomechanical filter was used to aerate water and remove fish waste. Each tank was illuminated with a 30-W fluorescent lamp on a 12:12 h light:dark photoperiod. Water temperature was maintained at $26 \pm 1°C$. We fed the guppies twice per day, alternating commercial food flakes and live brine shrimp nauplii *Artemia salina*. All subjects were experimentally naïve and spontaneously participated in the experiment. None of the subjects appeared to be stressed during the experiment, and after the experiment, they were kept for breeding only.

Apparatus and stimuli

We tested each subject in a $20 \times 50 \times 32$ cm glass tank containing 28 L of water (Figure 2). We placed 12 identical apparatuses in a dark room and used them at the same time. Each tank was shaped as an hourglass by means of 2 trapezoidal lateral compartments ($10 \times 6 \times 32$ cm) that were made of transparent plastic placed in the middle of the apparatus (Figure 2). The lateral compartments housed natural plants to provide an enriched environment for the subjects. All of the walls of each tank were covered with green
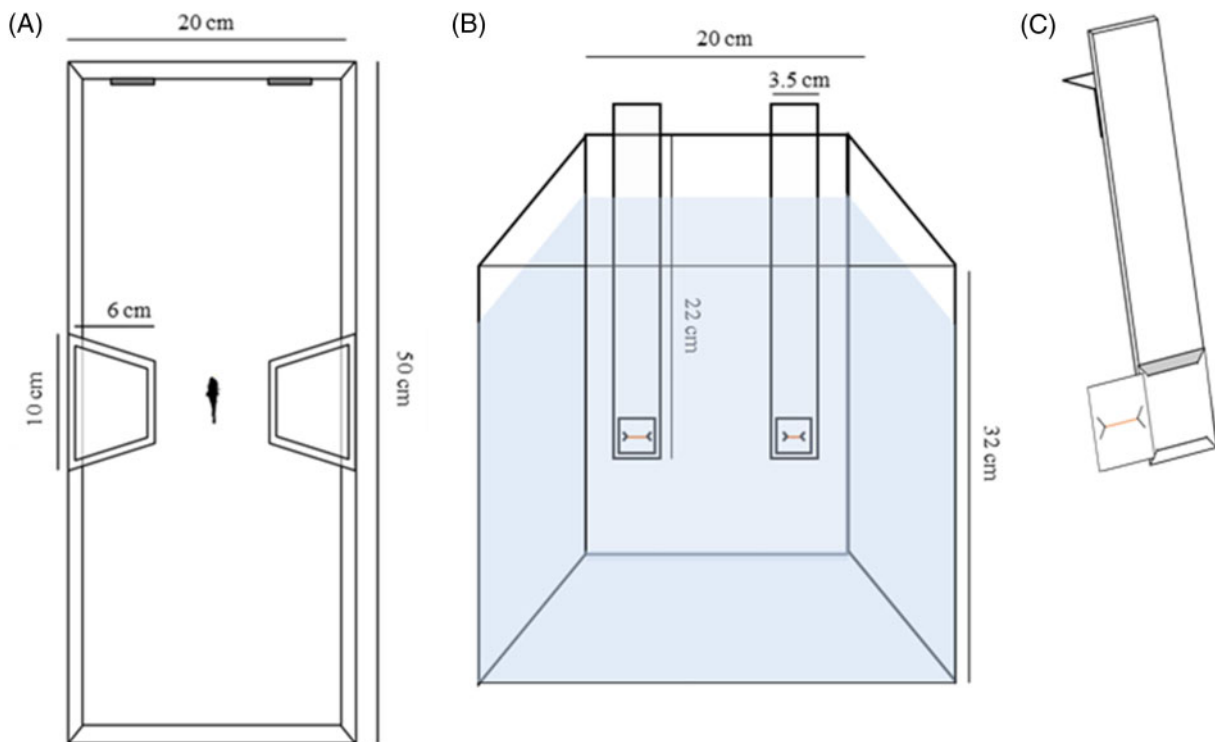

Figure 2. Experimental setup. Areal (**a**) and frontal (**b**) view of the experimental apparatus. Representation of a panel used to present the stimulus to the fish (**c**).

plastic. Therefore, the subjects could not see the experimenter, who could affect them during the experiment. One 15-W fluorescent lamp illuminated the apparatus (12:12 h light:dark cycle). We used orange target lines that were printed on white cards (3 × 3 cm) as stimuli. In fact, guppies have been shown to be highly attracted to orange stimuli (Rodd et al. 2002) and to perform better as a consequence (Lucon-Xiccato and Bisazza 2014; Gatto et al. 2017). In addition to orange lines, we printed black lines on the white cards as inducers (see below for details). To present the stimuli to the subjects, we affixed each card to the end of a transparent panel (3.5 × 15 cm) with an L-shaped blocker that allowed us to fix the panel on the wall of the tank. During each trial, we simultaneously presented 2 transparent panels on the same short wall of the tank.

Procedure
Each subject underwent pre-training, training, and test phases. We adapted a procedure that was previously used to investigate guppies' perception of another illusion, the Delboeuf illusion (Lucon-Xiccato et al. 2019).

Pre-training phase
We set up a 2-day pre-training phase to familiarize each subject with the experimental apparatus. During the first day of this phase, we inserted a card with only a central orange line into the tank 8 times (4 trials in the morning session and 4 trials in the afternoon session, with a 90-min interval between the sessions). After the subject approached the card, we used a Pasteur pipette to deliver a small food reward consisting of a drop of live brine shrimp *A. salina*, a highly preferred food item for guppies. No other food was provided during the entire experiment to maintain a high level of motivation during the trials. In each session, a minimum of 15 min separated 2 trials. We counterbalanced the short side of the tank in which the

stimulus was presented over the trials. On the second day, the subjects underwent 12 trials: 6 in the morning session and 6 in the afternoon session, with a 90-min interval between the sessions. We presented to the guppies a pair of transparent panels with 2 different length lines with no inducers (Different Length Control; Figure 3A). The ratio between the 2 lines was 0.67 (longer line: 3 cm in length; shorter line: 2 cm in length), a ratio that guppies had previously been shown to be able to discriminate (Bisazza et al. 2014; Miletto Petrazzini et al. 2015; Lucon-Xiccato et al. 2019). We expected the subjects to choose the stimulus with the longer orange line to get the food reward. If the subject approached the larger line, we used the Pasteur pipette to deliver the food while removing the transparent panel with the shorter line. If the subject approached the shorter line instead, the trial continued until the subject approached the longer line and the food reward was given ("correction" procedure). We counterbalanced the left/right positions of the longer line and the short side of the tank in which the cards were presented over the trials. We presented half trials on one short side of the tank and the other half trials on the other short side to avoid any spatial bias.

Training phase
In the training phase, each subject received 12 trials (6 in the morning session and 6 in the afternoon session with a 90-min interval between the sessions) per day for a maximum of 10 consecutive days. The guppies faced a discrimination task with 3 types of trials (Different Length Control, Outward Arrows Control, and Inward Arrows Control; Figure 3), which we presented according to a predetermined pseudo-random schedule. The Outward Arrows Control featured 2 different-length lines that both had black outwards-pointing arrows (Figure 3B), whereas the Inward Arrows Control also featured 2 different-length lines but with black inwards-pointing arrows (Figure 3C). In all 3 types of trials, the ratio
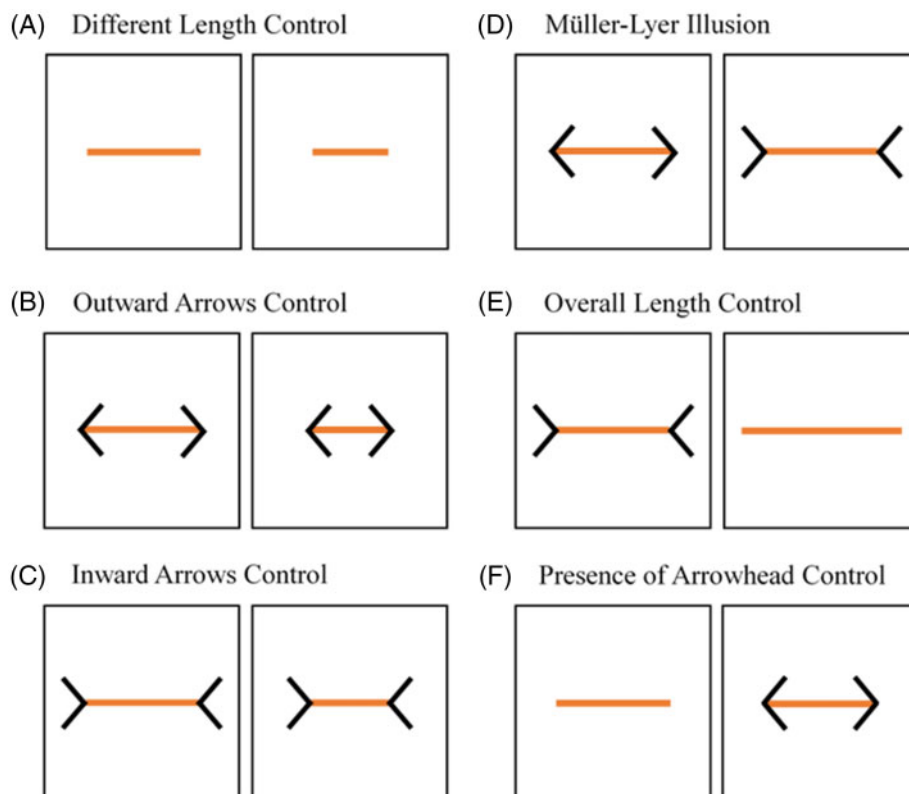

Figure 3. Stimuli. 2 white cards containing different or equal-length lines were presented: (**A**) Different length control, (**B**) outward arrows control, (**C**) inward arrows control, (**D**) Müller–Lyer illusion, (**E**) overall length control, and (**F**) presence of arrowhead control.

between the 2 lines was 0.67. We conducted the trials similarly to those of the second day of the pre-training phase with just one exception: if the subject approached the shorter line first, we removed both panels, and we did not provide a food reward (no correction was allowed). Each day, the subject saw each type of trial 4 times. We counterbalanced the left/right position of the longer line and the short side of the tank in which the cards were presented over the trials. Subjects could pass to the test phase if they met 1 of 2 learning criteria. The first learning criterion was defined as a rate of at least 70% correct choices (17/24) in the trials over 2 consecutive days (statistically significant for the Chi-squared test). The second learning criterion was defined as a statistically significant frequency of correct choices (at least 71/120) over 120 trials. The use of a double criterion is common in the literature in cognitive ethology (Byosiere et al. 2017, 2018; Miletto Petrazzini et al. 2018a); in particular, the same criteria were previously adopted in experiments involving this species (Miletto Petrazzini et al. 2018b; Lucon-Xiccato et al. 2019). Nine subjects did not reach either of the 2 learning criteria: they were not admitted to the test phase and were substituted with new subjects.

Test phase

In the test phase, each subject received 12 trials (6 in the morning session and 6 in the afternoon session, with a 90-min interval between the 2 sessions) per day for 10 consecutive days. The guppies faced a discrimination task with 6 types of trials, and we alternated the trials of each type according to a predetermined pseudorandom schedule. The subjects faced 20 trials per each type of trial for a total of 120 trials. Three types of trials (Different Length Control, Outward Arrows Control, and Inward Arrows Control) were the same from the training phase and represented the Control trials. Following the procedure that we laid out in the training phase, we administered the food reward in response to correct choices in these types of trials. In the other 3 types of trials, to avoid any bias, we did not provide any rewards. In particular, the fourth type of trial in the test phase comprised illusory trials that involved 2 same-length lines with different inducers, one with inwards-pointing arrowheads and the other with outwards-pointing arrowheads (Figure 3D). We expected the guppies to select the orange line that they perceived to be longer. However, the subjects could also use the physical differences between the 2 figures as a discriminative cue. In fact, the figure with the inwards-pointing arrowheads inevitably occupied an overall larger space than the one with the outwards-pointing arrowheads did. Therefore, in the test phase, we presented 2 additional types of trials: the Overall Length Control and the Presence of Arrowhead Control. The former consisted of the presentation of a line with the original inwards arrowheads paired with an overall equal-length line without any inducers (Figure 3E). We expected the guppies to select the isolated longer orange line if they based their size judgments in the illusory trials on the orange line's length and not on the stimuli's overall length. However, in this type of trial, the guppies could simply exhibit a spontaneous preference for choosing the line without any arrowheads. For this reason, we arranged the latter type of control trial: the Presence of Arrowhead Control. We presented the subjects with a line with outwards-pointing arrowheads that was paired with an overall equal-length line. Because the 2 orange lines were identical in length, we expected the guppies to randomly choose between the 2 lines if they did not have any bias in favor of the lines without arrowheads. All of the 6 abovementioned types of trials were successfully used in a previous investigation regarding dogs' perception of the Müller–Lyer illusion (Keep et al. 2018).

Statistical analyses

We analyzed the data in R version 3.5.2 (the R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). We performed a generalized mixed-effects model for binomial response distributions (GLMM, "glmer" function of the "lme4" R package) using the data on the choice of the longer line in the control trials with 2 different-sized lines (Different Length Control, Outward Arrows Control, Inward Arrows Control, and Overall Length Control), for the line with the outwards-pointing arrowheads in the Presence of Arrowhead Control or for the line with the inwards-pointing arrows in the illusory trials. The effect of the day was also tested in the model to ensure that guppies' performance was stable. Subsequently, all pairwise comparisons were performed with the Tukey honestly significant difference tests (Tukey 1949). As we found no significant difference among the Different Length Control, Outward Arrows Control, and Inward Arrows Control, such data were pooled and called "Summed Controls." The generalized mixed-effects model was re-performed with Summed Controls. Then, we used binomial tests ("binom.test" function) to compare the choice of the longer line in the control trials with 2 different-sized lines—for the line with the outwards-pointing arrowheads in the Presence of Arrowhead Control, or for the line with the inwards-pointing arrows in the illusory trials. The chance level was set at 0.50 both at the individual and at the group level. To assess inter-individual variation, we performed another generalized mixed-effects model. Cohen's d ("cohensD" function of the "lsr" package) was used as an effect size statistic. According to Cohen (1988), a Cohen's d of 0.2 represents a small effect size. A Cohen's d of 0.5 represents a medium effect size, and a Cohen's d of 0.8 represents a large effect size.

Results

Training phase

Ten out of 12 guppies passed to the test phase after meeting the learning criterion of a rate of 70% correct choices in trials over 2 consecutive days. On average, the fish needed 43.20 trials (SD = 25.44 trials) before meeting the learning criterion. The remaining 2 tested subjects reached the second learning criterion, achieving a significant frequency of correct choices over the total of 120 trials (binomial test: 77/120, $P < 0.01$; 74/120, $P < 0.05$). The Supplementary Table shows individual data of the training phase for all subjects, both the 12 subjects that passed to the test phase and the 9 that did not pass because they did not reach any learning criteria.

Test phase

The GLMM showed that the performance of guppies was stable across the 10 test days ($\chi^2_9 = 2.955$, $P = 0.966$) and varied as a function of the type of trials ($\chi^2_5 = 13.551$, $P < 0.05$). The day × type of trial interaction was not significant ($\chi^2_{45} = 40.894$, $P = 0.646$). The Tukey *post hoc* test revealed no significant difference among Different Length Control, Inward Arrows Control, and Outward Arrows Control (all $P > 0.264$). Therefore, the analysis was conducted pooling the data of these 3 controls. The GLMM confirmed a lack of effect of the day ($\chi^2_9 = 3.195$, $P = 0.956$) and an effect of the type of trial ($\chi^2_3 = 11.121$, $P < 0.05$). The day × type of trial interaction was not significant ($\chi^2_{27} = 13.931$, $P = 0.982$). The Tukey *post hoc* test revealed a significant difference between Summed Controls and the Presence of Arrowhead Control
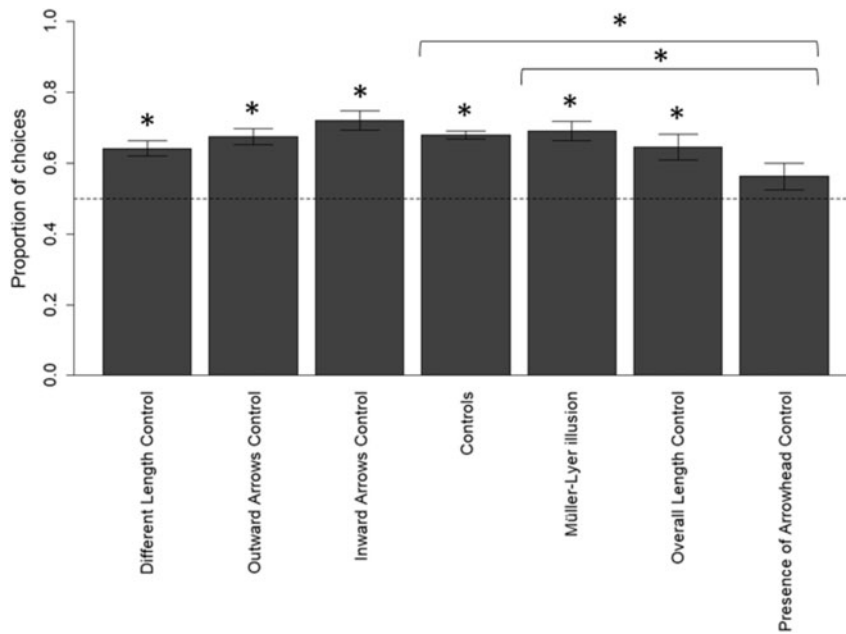
Figure 4. Results. The *Y*-axis refers to the proportion of choices for the longer line in the different length control, outward arrows control, inward arrows control, and overall length control; the proportion of choices for the line with the outward arrowheads in the presence of arrowhead control; and the proportion of choices for the line with the inward arrowheads in illusory trials. The asterisk (*) denotes a significant departure from the chance level (*P*<0.05). Brackets denote a significant comparison at Tukey *post hoc* test (*P*<0.05).

($P < 0.05$), as well as between illusory trials and the Presence of Arrowhead Control ($P < 0.05$). The guppies significantly selected the longer line in all 3 types of rewarded control trials: the Different Length Control (mean: 0.642, 95% CI [0.593, 0.690], $P < 0.001$, $d = 1.855$; Figure 4), Outward Arrows Control (mean: 0.675, 95% CI [0.627, 0.723], $d = 2.322$; Figure 4), and Inward Arrows Control (mean: 0.721, 95% CI [0.663, 0.779], $P < 0.001$, $d = 2.411$; Figure 4). In the illusory trials, the subjects exhibited a significant preference for the line with the inwards-pointing arrows (mean: 0.692, 95% CI [0.631, 0.752], $P < 0.001$, $d = 2.019$; Figure 4). This suggests that the guppies were sensitive to the Müller–Lyer illusion in the same direction as redtail splitfins and humans. However, before drawing such a conclusion, we needed to assess whether they were using physical differences, such as the overall stimulus size, between the 2 figures as the discriminative cue. Crucially, in the Overall Length Control trials, we found that the guppies chose the longer line (mean: 0.646, 95% CI [0.566, 0.725], $P < 0.01$, $d = 1.165$; Figure 4). One may argue that this result could be a consequence of a preference for the absence of arrowheads. In contrast with this hypothesis, they did not choose one stimulus more in the Presence of Arrowhead Control (mean: 0.563, 95% CI [0.480, 0.645], $P = 0.061$, $d = 0.481$; Figure 4). The GLMM also revealed no significant difference between the subjects ($\chi^2_{11} = 8.663$, $P = 0.653$) and no significant interaction between the subject $\times$ type of trial ($\chi^2_{33} = 34.734$, $P = 0.385$), showing that no inter-individual difference was detectable. Table 2 shows individual subjects' data.

Discussion

The aim of this study was to determine whether guppies perceive the Müller–Lyer illusion. In particular, the research's purpose was to test whether the spatial arrangement of inducers (i.e., inwards- or outwards-pointing arrowheads) influences guppies when they are

required to select the longer line between 2 lines. When tested with the illusory pattern, guppies significantly chose the line with inwards-pointing arrowheads, suggesting that they perceive the Müller–Lyer illusion in the same way as humans and other species, such as homing pigeons (Malott et al. 1967; Nakamura et al. 2006, 2009) and capuchin monkeys (Suganuma et al. 2007), do.

Testing animals in this type of task is quite challenging due to the communication barrier present. In fact, a crucial aspect of the investigations of the Müller–Lyer illusion involves the impossibility of instructing animals to attend to the target line only and to ignore the arrowheads in their size judgments. Although the animals were trained to select the longer target line regardless of the presence of arrowheads, they could have significantly chosen the line with ">< "ends in the illusory pattern because it is the longer overall figure. Such a possibility was recently discovered in the study of Keep et al. (2018), in which dogs were initially believed to perceive the Müller–Lyer illusion before control trials showed that they were using the global sizes of the 2 stimuli to solve the task. This prevented the researchers from drawing firm conclusions regarding dogs' perception of the Müller–Lyer illusion (Keep et al. 2018). In our study, we controlled for such a possibility with the Overall Length Control and the Presence of Arrowhead Control. When presented with a line with the original inwards-pointing arrowheads paired with an overall equal-length line without any inducers (namely, the Overall Length Control), the guppies selected the isolated longer line, suggesting that they were not using the overall length as the discriminative cue in the illusory trials. The Presence of Arrowhead Control condition, in which guppies did not select the line with outwards-pointing arrowheads significantly more than they did an overall equal-length line, reinforced this result. In fact, they demonstrated that they did not have any bias in favor of lines with or without arrowheads. All of these results clearly support the conclusion that guppies perceive the Müller–Lyer illusion as humans do, and together with the results of Sovrano et al. (2015), they

Table 2. Individual performance in the test phase (different length control, outward arrows control, inward arrows control and overall length control, summed controls: frequency of choices for the longerline; presence of arrowhead control: frequency of choices for the line with the outward arrowheads; illusory trials: frequency of choices for the line with the inward arrowheads)

Subject	Different length control	Outward arrows control	Inward arrows control	Summed controls	Müller–Lyer illusion	Overall length control	Presence of arrowhead control
1	12/20	14/20	16/20	42/60	10/20	15/20	11/20
	$P = 0.504$	$P = 0.115$	$P < 0.05*$	$P < 0.05*$	$P = 1.000$	$P < 0.05*$	$P = 0.824$
2	12/20	14/20	13/20	39/60	12/20	11/20	11/20
	$P = 0.504$	$P = 0.115$	$P = 0.263$	$P < 0.05*$	$P = 0.504$	$P = 0.824$	$P = 0.824$
3	11/20	15/20	13/20	39/60	16/20	12/20	10/20
	$P = 0.824$	$P < 0.05*$	$P = 0.263$	$P < 0.05*$	$P < 0.05*$	$P = 0.504$	$P = 1.000$
4	14/20	12/20	17/20	43/60	13/20	12/20	14/20
	$P = 0.115$	$P = 0.504$	$P < 0.05*$	$P < 0.05*$	$P = 0.263$	$P = 0.504$	$P = 0.115$
5	14/20	11/20	15/20	40/60	15/20	15/20	11/20
	$P = 0.115$	$P = 0.824$	$P < 0.05*$	$P < 0.05*$	$P < 0.05*$	$P < 0.05*$	$P = 0.824$
6	15/20	15/20	14/20	44/60	17/20	17/20	8/20
	$P < 0.05*$	$P < 0.05*$	$P = 0.115$	$P < 0.05*$	$P < 0.05*$	$P < 0.05*$	$P = 0.504$
7	13/20	14/20	11/20	38/60	15/20	15/20	10/20
	$P = 0.263$	$P = 0.115$	$P = 0.824$	$P = 0.052$	$P < 0.05*$	$P < 0.05*$	$P = 1.000$
8	11/20	13/20	13/20	37/60	15/20	11/20	12/20
	$P = 0.824$	$P = 0.263$	$P = 0.263$	$P = 0.092$	$P < 0.05*$	$P = 0.824$	$P = 0.504$
9	12/20	11/20	16/20	39/60	13/20	12/20	7/20
	$P = 0.504$	$P = 0.824$	$P < 0.05*$	$P < 0.05*$	$P = 0.263$	$P = 0.504$	$P = 0.263$
10	15/20	15/20	13/20	43/60	13/20	15/20	12/20
	$P < 0.05*$	$P < 0.05*$	$P = 0.263$	$P < 0.05*$	$P = 0.263$	$P < 0.05*$	$P = 0.504$
11	14/20	13/20	16/20	43/60	14/20	12/20	17/20
	$P = 0.115$	$P = 0.263$	$P < 0.05*$	$P < 0.05*$	$P = 0.115$	$P = 0.504$	$P < 0.05*$
12	11/20	15/20	16/20	42/60	13/20	8/20	12/20
	$P = 0.824$	$P < 0.05*$	$P < 0.05*$	$P < 0.05*$	$P = 0.263$	$P = 0.504$	$P = 0.504$
Mean proportion	0.642	0.675	0.721	0.679	0.692	0.646	0.563
95% confidence interval	0.593, 0.690	0.627, 0.723	0.663, 0.779	0.654, 0.704	0.631, 0.752	0.566, 0.725	0.480, 0.645

If guppies perceived the Müller–Lyer illusion as Redtail splitfins and humans, they were expected to select more than chance the stimulus with inward-pointing arrowheads ">< ."
Asterisks (*) denote a significant departure from chance level (0.5) at binomial tests.

suggest that the mechanisms underlying the perception of this illusion are shared across teleost fish and other vertebrates.

The results of group analyses are partially weakened by the individual performance analyses with binomial tests, where only 5 subjects clearly selected the line with the inwards-pointing arrows more than chance. This suggests that the illusion is weaker in guppies compared with humans. However, it is worth noting that most of the guppies that did not reach the threshold of a significant choice showed a non-significant trend for selecting the subjectively longer stimulus, thus leading to a robust significant choice at the group level. Such a conclusion is fully supported via a power analysis that revealed a Cohen's d of 2.019 ($d > 0.9$ is considered to be a "large effect size", see Cohen 1988). It is likely that the small proportion of guppies reaching a significant choice in binomial tests could be ascribed to the limited number of trials. Unfortunately, unlike monkeys or pigeons, fish cannot perform hundreds/thousands of trials due to the different metabolic requirements of cold-blooded vertebrates compared with warm-blooded species. Fish can be quickly satiated and might not search for food for prolonged periods, a fact that prevents the possibility of presenting hundreds of trials to guppies.

The inappropriate constancy-scaling theory (Gregory 1963) is the likely mechanism responsible for this illusory pattern. According to this theory, depth cues lead observers to interpret 2-dimensional figures as 3-dimensional images, evoking the constancy-scaling mechanisms for both size and shape. This theory proposes that the

"> <" ends resemble the perspective view of an inside corner, whereas the "< >" ends resemble the perspective view of an outside corner. In this way, the 2 target lines are perceived as being at different distances, and thus different sizes. In particular, in the "> <" configuration, the constancy-scaling mechanisms will increase the perceived length of the target line, whereas in the "< >" configuration, the mechanisms will decrease the line's perceived length. The constancy-scaling mechanisms could have great relevance for and a great impact on everyday life because size and distance estimations can be extrapolated from a 2-dimensional retinal image. The constancy-scaling mechanisms are reasonably present in the teleost fish species. A fish that does not possess constancy-scaling mechanisms could not use depth cues to judge the distances and therefore the sizes of certain objects. For example, a close and a distant fish might appear similar in size in the retina of such a fish. If a fish is not able to infer the size based on the perceived distance, it could not distinguish a distant big fish that could be a potential predator from a small close fish that might be prey. Fish that have constancy-scaling mechanisms could instead understand that the distant fish is bigger and therefore avoid such a fish.

Our results on guppies' perception of the Müller–Lyer illusion confirm those obtained for other teleost species, for example, the redtail splitfins (Sovrano et al. 2015), although we used a different experimental design and procedure from those used to obtain the other results. For example, we used food reinforcement for the training-shaping procedure, whereas Sovrano et al. (2015) adopted

social reinforcement. Even the choice modality was very different: whereas the guppies in our experiment had to approach the correct stimulus to get the food reward, the redtail splitfins had to open the correct door to rejoin their conspecifics by pressing on it with their snouts. Contradictory results can be found regarding the 2 teleost fish and the only cartilaginous fish tested for this illusion. In fact, bamboo sharks did not seem to perceive the Müller–Lyer illusion (Fuss et al. 2014).

Besides a possible effect of differences in their evolutionary history, different ecological explanations may account for such a discrepancy. For example, the constancy-scaling mechanisms might be more precise in guppies and redtail splitfins compared with bamboo sharks. In fact, whereas the 2 teleost fish are highly predated small fish, bamboo sharks are predators. The cost of misperceiving the size of a predator is reasonably higher than the cost of misperceiving the size of possible prey is in terms of survival. Indeed, prey risk being killed, whereas predators may risk eating smaller prey. Moreover, guppies and redtail splitfins are both diurnal species whereas bamboo sharks are nocturnal; therefore, they could have a different visual acuity. Further studies are needed to verify whether these or others interspecific ecological differences could explain the accuracy of constancy-scaling mechanisms. Alternatively, as several studies showed that the susceptibility levels to visual illusions largely vary as a function of the type of stimuli and procedures in the studies (Rosa Salva et al. 2013; Nakamura et al. 2014), the possibility exists that the difference reported between teleost and cartilaginous fish largely reflects the different procedures adopted. Indeed, the bamboo sharks that Fuss et al. (2014) tested could inspect the stimuli displayed on a screen very close to them, and they were trained to press their snouts against the positive stimulus to be rewarded with food.

To conclude, our results revealed that guppies can learn to distinguish between 2 different-length lines that differ by a ratio of 0.67, confirming guppies' ability to discriminate such a ratio (Bisazza et al. 2014; Lucon-Xiccato et al. 2019). Guppies are susceptible to the Müller–Lyer illusion, reinforcing the idea that humans' perceptual mechanisms underlying the extrapolation of 3-dimensional space through bidimensional retinal images are widespread among vertebrates. Whether this is due to the convergent evolution of perceptual systems among fish, birds, and mammals or inherited traits from a common ancestor remains, unfortunately, unknown.

Ethical note

This study complies with all laws of the country (Italy, D.L. 4 Marzo 2014, no. 26) in which it was performed, and it was done in accordance with the ethical standards of the institution or practice at which it was conducted (Protocol no. 13/2018).

Funding statement

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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