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At the root of numerical cognition: fish as a model species to study pre - verbal numerical abilities.

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1. INTRODUCTION

The study of numerical abilities represents one of the main issues of experimental psychology. The large body of evidence collected in literature agrees on the existence of two different types of abilities: verbal and pre-verbal numerical abilities (Gelman & Butterworth, 2005). The former abilities are strictly related to culture and language and permit us to learn the wide range of symbols and syntax required in school mathematics. However many studies also reported the existence of rudimentary numerical abilities that are independent from culture and language (Feigenson et al., 2004; Nieder, 2005). Such pre-verbal numerical skills are likely to have helped us to solve most of the quantity problems faced throughout human evolution, permitting to assess which group is more numerous even without verbal counting or mathematical syntax, for instance when we have to quickly evaluate which queue is less numerous at the airport.

Data supporting the existence of pre-verbal numerical systems come from four different field of psychology: cultural, cognitive, developmental and comparative psychology.

Cultural psychology investigates how cultural factors influence human behaviors and cognitive mechanisms. In our context, the study of numerical competence in cultures in which the language of numbers is either absent or limited represents an almost unique opportunity to elucidate the relations between language and arithmetic in adult humans. For instance, Pica and colleagues (2004) reported numerical abilities in native speakers of Mundurukú, a language that has number words only for the numbers 1 through 5. People belonging to this Amazonian population exhibit exact calculation in the range supported by verbal language, and approximate calculation beyond 5 units, showing that language plays a special role in the emergence of exact arithmetic but, at the same time, that language is not a *sine qua non* condition for rudimentary numerical abilities.

Cognitive psychology studies mental processes such as memory, problem solving, decision-making, perception, language use and attention. Despite it is difficult to disentangle verbal and pre-verbal numerical skills in adult humans, it is possible to investigate the mechanisms underlying pre-verbal numerical abilities by requiring participants to make a rapid judgments of relative numerosities (e.g., 150 milliseconds:

Agrillo & Piffer., 2012) while being prevented from using verbal counting (articulatory suppression). Using this procedure, it was demonstrated that adults required to estimate which set of dots is larger while repeating aloud non numerical words (i.e., abc) are still able to discriminate between quantities even without the possibility of counting the items

Developmental psychology focuses on human growth throughout the lifespan, from childhood to adulthood. This includes changes across a broad range of topics including physical, emotional, intellectual, social, perceptual and personality development. Interestingly, several studies have reported that pre-verbal human infants are able to discriminate between different number of objects, auditory sequences and actions (Xu & Spelke, 2000; Lipton & Spelke, 2003, Wood & Spelke, 2005) and that their accuracy increases over development, thus providing evidence that these skills emerge early in infancy, well before the acquisition of language, and persist throughout adulthood.

Comparative psychology studies the behavior and cognitive mechanisms of non-human animals and often involves comparing similarities and differences among species in order to understand their evolutionary relationships. Furthermore, comparative data can lead to a deeper comprehension of human psychology, trying to track continuities and discontinuities between human and non-human abilities. Evidence of similar cognitive abilities in different species then raises the question whether these traits evolved in a common ancestor or if they evolved independently as a result of similar selective pressures (Hauser & Spelke, 2004).

Although only our species has achieved high level of mathematical reasoning, numbers are not a human prerogative and rudimentary numerical abilities have been reported in non-human animals too (e.g., Uller, 2008; Feigenson et al. 2004). Indeed, there are many real-life situations in which the ability to discriminate between quantities is useful and there is no reason to believe that selective pressures in favour of the ability to quantify different magnitudes should have acted only on hominids. Quantitative abilities can permit animals to optimize foraging, enabling them to rapidly select the largest of two available sources of food (Beran, 2004). These abilities represent a powerful tool for anti-predator defence, reducing the probability of being spotted by predators (Agrillo et al., 2012; Gòmez-Laplaza & Gerlai, 2011a, b) and are also useful in social interactions: for instance, lionesses and hyenas are more willing to enter a contest when their group outnumbers that of opponents (McComb et al., 1994, Benson-Amram et al., 2011).

The discovery that the capacity to discriminate between quantities is widespread among vertebrates led several authors to suggest that these non-symbolic numerical systems may have a long evolutionary history (Beran, 2008a; Feigenson, et al., 2004; Agrillo et al., 2012).

Especially in the last decade, researchers have increased their attention on numerical cognition. Part of this remarkable development is related to the discovery of a linkage between verbal and pre-verbal numerical systems. Several lines of research suggest that our verbal numerical abilities are rooted in pre-verbal numerical systems (Halberda et al., 2008; Park & Brannon, 2013). For instance it has been found that deficits in the study of mathematics, such as dyscalculia, are associated with low performance in non-verbal numerical tasks (Piazza et al., 2010; Furman & Rubinstein, 2012). In this sense, the study of pre-verbal numerical abilities becomes crucial to understand the foundation of our mathematical abilities.

With respect to this topic, animal models can play a key role in increasing our knowledge on human cognitive systems, as in other research fields, helping to investigate the exact mechanisms underlying pre-verbal numerical abilities. Actually there are several advantages in using animals models in this issue: the study of non-human animals permit to investigate numerical systems without any influence of language and culture; some species (such as zebrafish or drosophila) represent an ideal model to investigate the genetic origin of these cognitive systems, as we now have full knowledge of their genome. Also, rapidly growing species represent proper models to study the ontogeny and developmental trajectory of pre-verbal numerical abilities, as well as the role of experience and maturation can be finely manipulated in controlled laboratory studies with non-human animals.

1.1. Numerical abilities in animals

1.1.1. Field studies

Being able to discriminate between different quantities yield benefits in several contexts. For example, a number of studies have shown that different species decide whether attack intruders or retreat depending on the number of individuals in the interacting groups.

McComb and colleagues (1994) investigated whether lionesses (*Panthera leo*) in the Serengeti National Park based their aggressive interactions on the assessment of the relative number of intruders. Researchers used a play back technique to reproduce roar recordings from one single stranger lioness or from a group of three females to simulate the presence of unfamiliar individuals. Lionesses were more likely to approach the playbacks when they outnumbered the opposite group.

Recently a similar behavior has been reported in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve using a playback procedure similar to the one previously adopted by McComb and colleagues (1994). Hyenas were more vigilant when they heard contact calls from three unknown individuals than from two or one and took more risk in approaching the speakers when they were in numerical advantage (Benson-Amram et al., 2011) (Fig. 1).

Fig. 1: *Hyenas tend to attack other conspecifics when they outnumber the potential opponents (Benson-Amram et al., 2011)*

Brood parasitism offers another interesting ecological context in which numerical assessment play a key role in decision making. The number of eggs in a nest is an indicator of the nest stage and its quality. A small clutch size indicates that the host has not begun the incubation yet and hence parasitic individuals can lay their own eggs to synchronize hatching with that of the host. The number of eggs may also affect the efficiency of incubation: a large number reduces the probability of hatching and sometimes the nest may be abandoned because of the lower hatching success. Since the reproductive success in brood parasitic species is determined by the number of eggs in the host nest, it is likely that selective pressure has favoured mechanisms to discriminate quantities.

White and colleagues (2009) studied the ability of an obligate brood parasite, the brown-headed cowbirds (*Molothrus ater*), to assess the number of eggs in possible host nests in order to lay in the most suitable one. Cowbird host usually lays one egg per day until it reaches the clutch size and then starts the incubation. Researchers manipulated the number of eggs in two experimental nests, adding one egg each day in one nest and adding a fewer number of eggs in the other one for a different number of days. Data showed that female cowbirds used both the total number of eggs in the nests and the rate at which eggs were added as indicator of the nest stage in order to choose the one in which the number changed in accordance with the number of days elapsed between two successive visits. Comparable results have been obtained with a conspecific brood parasite, the wood duck (*Aix sponsa*) (Odell & Eadie, 2010). Four kinds of experimental nests containing a different number of eggs were used to simulate different nest stages: 5 eggs (reproducing a situation in which the host was still laying), 10 eggs (the host was close to start the incubation), 15 eggs (incubation started), 20 eggs (incubation started or nest abandoned). Parasite females preferred to lay eggs in the nests with the smaller clutch size (5 or 10 eggs), regulated the number of eggs laid on the basis of the eggs already present and were more willing to incubate the experimental nests with 5 or 10 eggs.

Animals rely on quantitative information also to guide their foraging decisions. Theories of optimal foraging (MacArthur & Pianka 1966; Stephens & Krebs 1986) predict that animals have evolved strategies to maximize their energy intake when foraging, and hence it is expected they have evolved the ability to select larger amounts of food.

Different avian species have shown to prefer the larger quantity of food in natural environment. Hunt and colleagues investigated in the Karori Wildlife Sanctuary whether the ability of New Zealand robins (*Petroica australis*) to retrieve cached food was based on the numerical estimation of hidden prey. Different number of mealworms were sequentially placed in two artificial cache sites and then obscured from robins' view. In this way subjects could never see the whole content of each group, thus preventing the use of non-numerical information (see Section 1.2). Even in this condition, robins chose the larger amount of food (1 vs. 2, 2 vs. 3, 3 vs. 4 and 4 vs. 8). Furthermore, when subjects were allowed to retrieve only a fraction of the prey because a certain number of mealworms was removed after being shown to birds, subjects searched for longer the expected number of prey, showing the ability to take into account quantitative information (Hunt et al., 2008). Garland and colleagues (2012) observed a similar performance in North Island robins (*Petroica longipes*), both when mealworms were sequentially hidden and when prey were simultaneously presented as a whole set.

In conclusion, field studies show that the ability to discriminate between quantities can provide advantages for animals' fitness and hence it is plausible that multiple selective pressure have acted on numerous species to cope with different problems in their natural environment.

1.1.2. Laboratory studies

Field studies are useful to understand the ecological relevance of numerical information. However, they seldom control for non-numerical cues, making it difficult to understand whether animals use numerical information only or other perceptual cues. With respect to this topic laboratory researches permit to study numerical abilities under more controlled conditions. Two procedures are commonly used: the spontaneous choice paradigm and the training procedure.

The first method investigates the spontaneous preference for small or large quantities based on relative numerousness judgements (e. g., A is greater than B). This ability represents a basic discrimination mechanism that does not necessary require real enumeration.

Food has been commonly used as stimulus due to its high ecological value for animals. Since it is more advantageous to select larger amounts of food, they are expected to spontaneously choose the larger group of food items.

Red-backed salamanders (*Plethodon cinereus*) placed in a T-shape enclosure with two plastic tubes containing a different number of fruit flies, approached the one with the larger number of prey in 1 vs. 2 and 2 vs. 3 numerical contrasts but not when the choice was between 3 vs. 4 or 4 vs. 6 fruit flies (Uller et al., 2003) (Fig. 2).

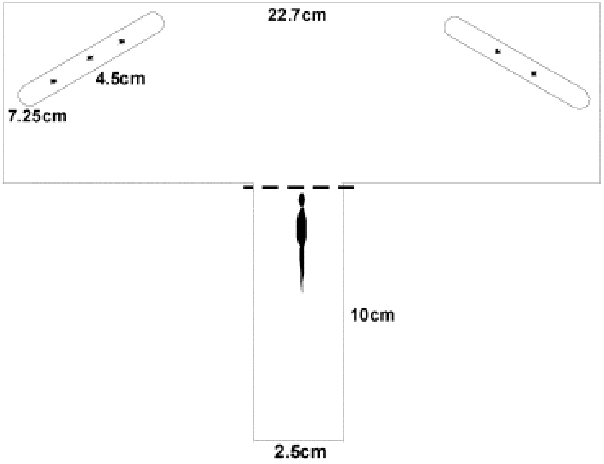


Fig. 2: *Schematic representation of the experimental set-up used by Uller and colleagues (2003). Salamanders selected the larger number of fruit flies in 1 vs.2 and 2 vs.3.*

Recently, Perdue and colleagues (2012) investigated relative quantity judgements in two African elephants (*Loxodonta africana*). In the Visible condition, subjects could choose between two sets of food items (from 1 through 10) presented simultaneously as whole sets. In the Nonvisible condition, food items were inserted sequentially in two containers to avoid animals from seeing the entire sets and compare them directly. The elephants chose the larger amount of food in both conditions but their performance decreased as the ratio between the two quantities increased becoming more difficult.

Other studies have showed that animals as diverse as dogs (Ward & Smuts, 2007), sea lions (Abramson et al., 2011), beluga whale and bottlenose dolphins (Abramson et al., 2013) select the larger quantity of food.

In other works, social companions were used as stimuli (Agrillo et al., 2008a; Gòmez-Laplaza & Gerlai, 2011 a, b). The assumption was that social animals can take advantage in nature by spontaneously joining the larger group of conspecifics to reduce the risk of being caught by predators. For example, it has been shown that different fish species prefer to join larger groups of conspecifics when placed in an unfamiliar and potentially dangerous environment to reduce the chance of being spotted by predators (fathead minnow: Hager & Helfman 1991; banded killifish: Hoare et al., 2004; green swordtail: Buckingham et al., 2007).

Despite quantity discrimination has been mainly investigated in vertebrates, recently, it has been shown that some invertebrates display rudimentary quantity abilities too. Carazo and colleagues (2009) exposed male yellow mealworm beetles (*Tenebrio molitor*) to substrates bearing odour from groups of females differing in number (1 vs. 4, 1 vs. 3, 2 vs. 4 and 1 vs. 2). The subjects discriminated between the sources of odour reflecting 1 vs. 4 and 1 vs. 3 (0.25 and 0.33 ratios respectively) females, while no choice was observed for 1 vs. 2 or 2 vs. 4 contrasts (0.5 ratio).

For what concern training procedures, subjects are commonly required to learn a numerical rule in order to receive a reward.

Spontaneous choice tasks can be run quickly but they usually require a relatively large sample size, making the study of some uncommon species (e.g., marine mammals, primates) more difficult. For this reason, training procedures may be the best way for testing a limited number of individuals multiple times in order to collect sufficient data, even though this kind of procedure sometimes may require time consuming efforts.

Early laboratory studies were performed by Otto Koehler who tested different avian species (pigeons, jackdaws and budgerigars). His studies suggested that birds were able to discriminate groups of items presented simultaneously and to assess the number of events that occur successively (Koehler, 1941; 1951).

Despite Koelher’s researches were criticized for lack of control, further studies using computerized tasks confirmed his findings. Emmerton and Delius (1993) trained pigeons (*Columba livia*) to discriminate between arrays of white dots on a dark background using a standard operant procedure. Stimuli varied in number and dot size across trials and when pigeons chose the correct array (the large one), they received a food reinforcement, whereas the choice of the wrong quantity led to a period of darkness. In transfer trials with new pairs of stimuli equated for brightness, pigeons were capable to discriminate up to 6 vs. 7 dots but their performance decreased to chance level in 7 vs. 8. Other studies have reported that pigeons are also able to order serially numerical quantities (from 1 through 7) (Emmerton et al., 1997), and to associate a specific number of pecks in response to visual symbols representing numbers from 1 to 6 (Xia et al., 2000).

Brannon and Terrace (1998) trained two rhesus monkeys (*Macaca mulatta*) to order numerosities from 1 to 4 in ascendant way. Stimuli were groups of two-dimensional figures varying in size, shape, color and surface area to avoid monkeys from using non-numerical cues rather than number to order the numerosities. When the subjects learned the ascendant rule (1-2-3-4), new stimuli with novel numerosities (5-9) were introduced in transfer test to see whether monkeys were able to generalize to unfamiliar numerosities. Both animals correctly ordered the sequence from 1 to 9 showing to be able to represent the ordinal relations among the numerosities. The same paradigm was successfully adopted in studies with capuchin monkeys (*Cebus apella*) (Judge et al., 2005) and pigeons (Scarf et al., 2011), revealing a similar pattern of choice.

More recently, american black bears (*Ursus americanus*) have been trained to discriminate between groups of dots (from 1 to 10) in three conditions (Vonk & Beran, 2012). In the first one, the dots were static, in the second condition, the items moved on a screen and in the third one, bears had to discriminate subsets of dots within each group of moving items. All the subjects successfully chose the correct set of items in all three conditions even though the performance decreased with ratio and was less accurate with moving stimuli. The lower performance in enumerating moving dots could be due to the fact that the black bear is a non-social species and hence it probably does not need a precise mechanism to track individual members of the moving group (see Section 1.3). However, since they were able to discriminate the moving sets differing in numerosity to some extent, it is possible they have evolved a less accurate system to quantify amount of moving objects.

Insects have been trained to study their numerical skills too. Bees (*Apis mellifera*) were trained to obtain a sugar solution (a food reward) after they had flown beyond a certain number of

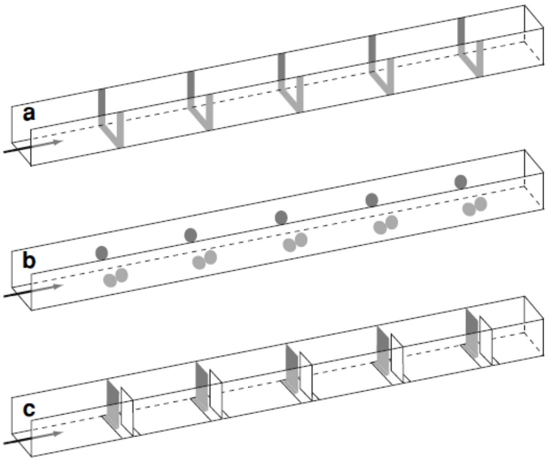


Fig. 3: *Experimental tunnels with different landmarks spaced at regular intervals to assess numerical ordering abilities in bees (Dacke & Srinivasan, 2008)*

regularly spaced landmarks in a tunnel. When the distance between the landmarks was modified to verify whether bees had learned to use the exact number of landmarks passed instead of the distance covered, they used the numerical position to receive the reward rather than their spatial arrangements. When subjects were presented with novel landmarks (Fig. 3), bees were still able to identify the correct one on the basis of its numerical location (Dacke & Srinivasan, 2008).

1.2. The role of continuous variables in quantity discrimination judgments

Quantity judgements are not always necessarily “numerical” judgments. The discrimination between “more” or “less” items could be achieved without counting. In natural environment numerosity normally co-varies with other perceptual cues, usually named continuous variables or quantities (i.e., density, overall space occupied by the items, brightness, etc.), that may be informative about the magnitude of the sets to be compared. For instance, two groups with a different number of apples normally differ in the overall space occupied, in the their total volume and cumulative surface area. As a consequence animals can rely on continuous quantities to guide their choice instead of using numbers.

Some authors suggest that animals spontaneously represent number and can discriminate sets of items on the basis of their numerosity (Gallistel & Gelman, 1992; Dehaene, 1997).

Conversely, other authors claimed that animals are not naturally attuned to number since numerical information is less salient in natural contexts than physical attributes. Furthermore they argue that number is more cognitively demanding to be processed than perceptual cues and hence animals would mainly attend to continuous quantities than discrete ones. However, when no other cues are available animals could base their choice on number as a “last resort” strategy (Breukelaar & Dalrymple-Alford, 1998; Davis, 1993; Davis & Memmot, 1982; Davis & Perusse, 1988).

Numerosity is a property of a set of items: it means that numerosity remains constant even when continuous variables are manipulated and it changes only if other items are added or subtracted. In this sense, if a subject still discriminates groups containing different number of objects when perceptual features are controlled it probably means that the subjects can use numerical information.

Different experimental strategies have been used in literature to disentangle the relative salience of numerical and non-numerical information.

One involves the use of stimuli controlled for continuous quantities (i. e., cumulative surface area is equated between the sets) in order to assess whether subjects can use numerical information only (Vonk & Beran, 2012; Emmerton & Renner, 2006; Brannon & Terrace 1998; Rugani et al., 2008; Xu & Spelke, 2000, Clearfiled & Mix, 1999).

The other strategy, commonly called item-by-item procedure, consists in sequentially presenting each item in a set in order to prevent the subjects from having a global view of the entire sets. For example, the experimenter place 3 apples in one opaque container and 2 apples in a second one, and then the subjects is allowed to select only one container. Furthermore, since the numerosity is correlated with the amount of time required for hiding the items, total duration and ratio of presentation are also controlled. In this conditions it is difficult that subjects rely on continuous quantities and the only way to solve the task is to mentally add each item within each container and then compare the two representations (Hauser et al., 2000; Perdue et al., 2012; Utrata et al., 2012, Feigenson et al., 2002a).

To date, there are conflicting data about the salience of numerical information both in human and animal literature.

For instance, when chimpanzees (*Pan troglodytes*) were given the choice between arrays containing a different number of crackers, presented both simultaneously and sequentially, they chose the set with the larger amount of food even when it contained the smallest number of food treats. Subjects based their preference on the total amount rather than on the number probably because animals naturally try to maximize their energy intake and the total amount is usually a better indicator of the total edible mass than the number of food items (Beran et al., 2008a).

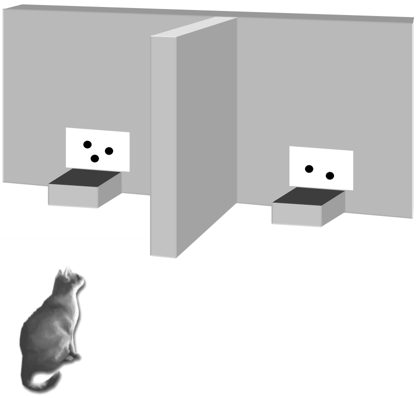


Fig. 4: *Representation of the experimental set-up used to study numerical cognition in cats (Pisa & Agrillo,2009).*

Likewise, Pisa and Agrillo (2009) observed that the ability to discriminate quantities is strongly affected by continuous quantities in cats (*Felis silvestris catus*). Four cats were trained to discriminate between 2 and 3 black dots on a withe background in order to get a food reward (Fig. 4). Stimuli used during the training phase were not controlled for continuous variables and the dots in the larger set had also the largest cumulative surface area and occupied the largest space. Cats learned the discrimination in the training phase but their performance dropped to chance level during the test phase when new stimuli controlled for cumulative surface area were presented, suggesting that they were attending to non-numerical cues to discriminate between quantities.

When biologically relevant stimuli are used, such as living organisms, the amount of movement is another cue that may play an important role in decision making. Larger groups usually have an higher level of activity providing clear information about the group size. Despite Uller and colleagues (2003) have shown that salamanders chose the larger amount of fruit flies, they did not control for the total movement of the prey. Since salamanders are very sensitive to the movement pattern and the speed of the prey, Krusche and colleagues (2010) used a two choice task to study whether salamanders could assess differences in large groups (8 vs. 16 crickets) even when the movement was controlled. In short, salamanders were presented with three sets of stimuli: live crickets, videos of live crickets or computer-animated crickets. The latter kind of stimuli allowed to control for perceptual cues that co-varied with the number of the prey (movement and distribution). Salamanders preferred the larger group when they could choose between live crickets and videos of live crickets but they chose randomly when the movement duration was equal in the computer-animated stimuli. This result demonstrated that salamanders were not sensitive to number but rather that the total activity of the stimuli was a prominent cue in their decision making.

All together, these studies bolster the last resort hypothesis showing a preferential use of continuous rather than discrete (numerical) variables in different species.

After all, it is remarkable that continuous quantities are relevant in humans too. For instance, some authors claim that human infants attend preferentially to continuous variables than to numerical information to discriminate between quantities. Despite in early studies infants proved be able to discriminate between small number of items (Starkey & Cooper, 1980; Strauss & Curtis, 1981; Antell & Keating, 1983), continuous quantities were correlated with number and therefore it is possible that infants responded to changes in these cues instead of changes in numerical information. Clearfield and Mix (1999) used the habituation procedure to study whether infants were able to detect a change in number or a change in continuous quantities in a number discrimination task. Six month-old infants were initially presented with sets of two or three bi–dimensional black squares with constant contour length until they reached the habituation criterion. Subsequently, stimuli familiar in number but novel in contour length and stimuli familiar in contour length but novel in number were shown alternatively in test phase. Infants looked longer at the arrays with new contour length than at the sets novel in number thus suggesting that they were able to detect changes in continuous variables but not in number. A similar conclusion was reached by Feigenson and colleagues (2002a) in a study where 10- to 12-month-old infants were allowed to choose one of two containers where a different amount of crackers had been sequentially inserted. When the number of crackers co-varied with the cumulative surface area and hence with the total amount of edible food, infants preferred the larger group in 1 vs. 2 and 2 vs. 3 comparisons. However, when the crackers were of different sizes, the choice was determined by total surface area and the authors concluded that infants relied on continuous variables rather than on numerical information.

Continuous quantities affect numerical estimation also in adult humans who have been shown to spontaneously and automatically represent number, size and area when judging which array of dots is larger. Continuous variables were found to interfere with numerosity judgements more than variation in number interfered with area judgements. Subjects relied on number only when the numerical distance between the sets was large, but when the distance was small they spontaneously based their choice on the area of the dots showing to be unable to ignore area/size information (Hurewitz et al., 2006).
Gebuis and Reynvoet (2012) have proposed that we normally make quantity judgements by weighing different visual cues because in daily life number usually co-varies with

perceptual cues and this is likely to have favoured the evolution of systems that rely on multiple information. In their study, adults were required to estimate the number of dots in arrays differing in numerosity (12, 20, 28, 36 or 44). The visual properties of the stimuli were manipulated in order to be not predictive of the numerosity and hence there was no correlation between number and continuous variables (i.e: the cumulative surface area in the 28-dot set was smaller than in 20-dot set but larger than in the 36-dot one). Subjects unconsciously estimated the number on the basis of the visual cues. This result bears on the hypothesis that we are probably unable to process number independent of the perceptual cues.

However data are controversial since numerous studies have reported a spontaneous use of numbers both in humans and non-human animals thus challenging the last-resort hypothesis.

Cantlon and Brannon (2007a) showed that rhesus monkeys were able to naturally extract numerical information. Monkeys were trained to discriminate between arrays containing a different number of two-dimensional figures using a match-to-sample technique. During training stimuli were not controlled for continuous variables and monkeys could use both continuous and discrete quantities to solve the task. However, when continuous quantities were pitted against number in test phase, all three number-experienced monkeys matched the stimuli on the basis of numerical information when the numerical ratio was easy. In addition authors tested also a number-naïve monkey to verify how much the training history might have influenced the performance of monkeys that had undergone extensive number training. Even though the monkey without previous experience on number relied more on continuous cues when the numerical ratio was difficult, it spontaneously used number to solve the task when the ratio between the stimuli was small.

Animals other than primates are capable of encoding numerical information. Domestic chicks and pigeons successfully discriminated the larger set of geometrical figures both when continuous and discrete quantities were simultaneously available and when continuous quantities were controlled (overall area and contour length in chicks: Rugani et al., 2008; brightness and area in pigeons: Emmerton & Ranner, 2006), showing a similar performance in the two conditions.

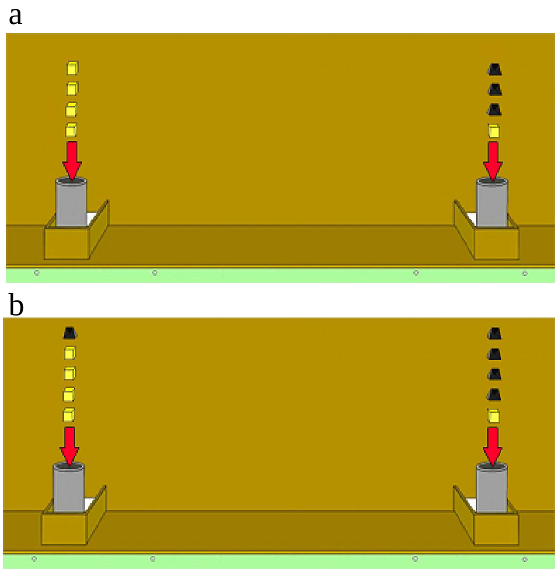


Fig. 5: *Control experiment to verify whether wolves base their choice on continuous cues: Time control (a), Stone control (b). (Utrata et al., 2012)*

In a recent study by Utrata and colleagues (2012) wolves (*Canis lupus*) chose the larger set of food treats (1-4) sequentially introduced into two opaque containers, even in control tests set up to rule out the possible influence of continuous quantities. In the time control experiment (Fig. 5a), stones were added in the smaller set in order to equate the amount of objects hidden and the handling time. In the stone control experiment (Fig. 5b), an extra stone was added in both sets to exclude that wolves simply have avoided the sound of the stone in the smaller group in the previous control.

Wolves successfully discriminated between the two numerosities, suggesting that their preference was based on the effective number of food items.

Taken together, these results revealed a number sense in non-human animals and a natural sensitivity to numerical information.

A similar conclusion was drawn about human infants. Six-month old infants proved be able to discriminate 16 from 8 dots when stimuli were controlled for continuous variables showing to be capable of detecting a twofold change in number (Xu & Spelke, 2000). More recently Cordes and Brannon (2008) have provided data challenging the hypothesis that the representation of continuous quantities is less complex than the representation of number in human infants. Six-month-old infants were initially habituated to dot sets with constant cumulative surface area but with different number of dots to make numerical information irrelevant. In test phase stimuli that alternated between the familiar and a novel cumulative surface area were shown. Infants failed to detect a twofold and a threefold change in area and required at least a fourfold change to notice the difference. These results are clearly in contrast with the idea that pre-verbal infants spontaneously attend to continuous quantities and use number only as a last resort strategy. Furthermore it seems that continuous variables are more difficult to be processed, at least when arrays with multiple elements are presented.

Interestingly Nys and Content (2012) have shown that number is as salient as area in a discrimination task in adults. Participants were presented with dot arrays (30 through 100) and were asked to perform a number comparison task or an area comparison task. Stimuli were both congruent (number and area were correlated) and incongruent (number and area were negatively correlated). Results showed that cumulative area affected the ability to compare set differing in numerosity, thus confirming previous data (Hurewitz et al., 2006). Interestingly, numerosity interfered with area judgements providing evidence that adult spontaneously extracted number. Overall, the fact that adults could not ignore numerical information when processing continuous quantities supports the view that number is a prominent cue.

Even though data on the relative salience of continuous variables on number and *vice versa* are very ambivalent, it seems to be widely confirmed that the redundancy of information may facilitate to solve quantity discrimination tasks both in humans and in non-human animals.

For example, Agrillo and colleagues (2011) trained mosquitofish to discriminate 2 dots from 3 in order to re-join conspecifics in an external tank, in three conditions. In the first one stimuli were controlled for continuous quantities and subjects could use only numerical information; in the second one the number was kept constant (1 vs. 1) and only continuous quantities were available (the area in one stimulus was 2/3 the area of the other one) and in the last condition both number and continuous quantities were available. Fish learned to discriminate more quickly in the last condition compared to when they could use continuous quantities only or numbers only; interestingly, no difference in the learning rate between these two latter conditions was found. Hence, the authors suggested that the redundancy of information helped fish in learning faster to solve the task and that processing numbers was not more cognitively demanding than processing continuous quantities.

Among adult humans, multiple information facilitate learning and memory either presented in the same sensory modality or in different modalities (Armelius & Armelius, 1974; Neil et al., 2006). Evidence for the use of multiple types of information has been reported for infants too (Suanda et al., 2008). Iuculano and colleagues (2008) found that 8- to 9-year-old were more accurate when discriminating stimuli in which both area and number suggested the correct response than stimuli in which only area or only number were available.

In a study by Jordan and colleagues (2008a) 6-month-old infants were habituated to see a movie in which a ball dropped and bounced 8 or 12 times in three different conditions. In the first one every time the ball bounced, it produced a tone synchronized with each ball impact, in the second condition infants could see just the ball bouncing without any sound and in the third situation classical music was played all along the duration of the visual sequence thus not providing synchronous numerical information. During test phase sequences with the ball bouncing 8 and 12 times were shown alternately in all three conditions. Infants looked longer at the novel sequence only when they could see the ball and listen the tone synchronized with the impact, providing the first evidence of 6-month-old infants' ability to discriminate a 2:3 ratio: a level of discrimination normally achieved at about 9 months of age when unimodal stimuli are used (Xu & Spelke, 2000, Lipton & Spelke, 2003). The authors claimed that redundant information across multiple sensory modalities might have improved infants' accuracy in a quantity discrimination task.

1.3. One or multiple numerical systems of numerical representation ?

Numerous studies have provided evidence that humans have both verbal and pre-verbal numerical systems to represent and compare quantities. The verbal systems are unique to humans and are promoted by teaching and enhanced by day to day engagement in numbers (Carey, 2004; Feigenson et al., 2004). They allows complex mathematical reasoning and are strictly related to language since imply the use of abstract numerical symbols (namely, Arabic words and number words) to perform arithmetic calculus. Through the verbal systems we can discriminate precisely both large and small quantities, that is, the discrimination between 150 and 155 items is as much accurate as the discrimination between 7 and 8.

On the other hand, we are also equipped with pre-verbal numerical systems that allow to quickly estimate which group is larger without symbols or verbal counting, for instance when we have to select which queue is shorter at the ticket office. However quantity discrimination is less accurate when pre-verbal number systems are involved.

In last years a number of studies have found a positive correlation between our complex numerical skills and pre-verbal quantification systems: the more accurate we are in non-verbal numerical estimation, the better we are in mathematics (Halberda et al., 2008; Lyons & Beilock, 2011). Furthermore children's acuity in quantity estimation tasks measured at preschool predicts their performance on math achievement (Mazzocco et al., 2011). It is also worth noting that Park and Brannon (2013) have observed that improvement in a non-symbolic-arithmetic task after a specific training transferred to improvement in a symbolic-arithmetic task thus providing the first evidence that pre-verbal systems may be causally related to verbal systems. Overall, these studies support the hypothesis that pre-verbal numerical systems underlie our mathematical abilities.

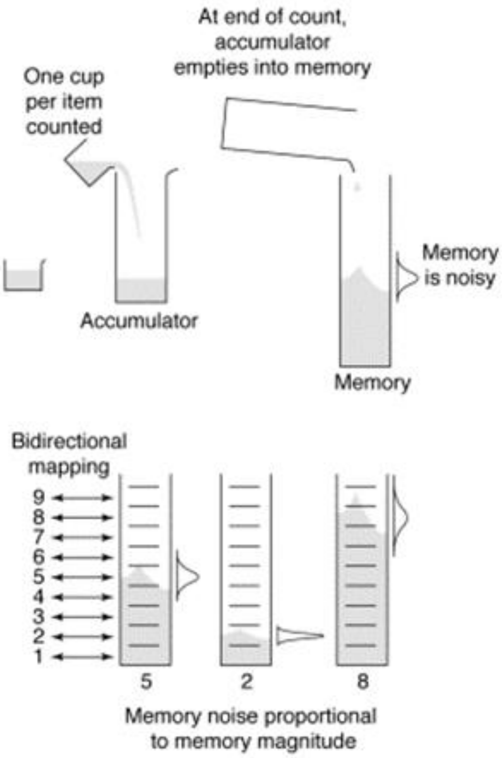


Fig. 6: *Depiction of the accumulator mechanism proposed by Meck and Church (1983). The mechanism generates an impulse for each object in a set. The magnitude accumulated at the end of the count represents the total number of items.*

Regarding the pre-verbal systems, many authors agree with the existence of an approximate number system (ANS) able to support the representation of numerosities without language. The pre-verbal counting process could be explained by the accumulator model proposed by Meck and Church (1983). The system works by generating an impulse for each object in a set: the more items to be enumerated, the more impulses are generated and stored in a mental accumulator. The final magnitude of impulses in the accumulator at the end of the counting process is stored into memory and represents the cardinal value of the counted array (Fig. 6). This system does not represent individual objects but rather, the numerosity of a set is represented as an approximate mental magnitude proportional to the quantity to be represented. As a consequence, if the difference between two sets is small, their representations may overlap, leading to lower accuracy.

The numerical discrimination through this mechanism is subject to Weber's law which states that the just-noticeable difference between two stimuli is proportional to the magnitude of the stimuli. It means that discrimination depends on the ratio between two quantities to be compared rather than on their absolute difference (Gallistel & Gelman, 1992): the discrimination between 10 from 20 (ratio 1:2) is easier than the discrimination between 40 from 50 (ratio 4:5) even if the numerical distance is the same (10 units). For this reason as numerical magnitudes increase, a larger numerical distance is required to discriminate between quantities. Despite the discrimination is not accurate and is ratio dependent, this system allows quantity discrimination without an apparent upper limit.

When adults are tested in quantity discrimination tasks which prevent verbal counting, their performance is commonly affected by the ratio confirming the idea of a pre-verbal number system that underlies quantity discrimination without symbols.

For example, Cordes and colleagues (2001) provided data showing a difference in accuracy between the verbal and non-verbal numerical systems. Adult humans were asked to press a key as many times as indicated by an Arabic number appeared on a screen. In one condition subjects had to repeat the word “The” every time they pressed the key in order to prevent them from counting; conversely in the other condition subjects could count out loud at each press. The number of errors in pressing the key until reaching the target number was greater when people could not verbally count. Furthermore the number of key presses and the variability in the responses were proportional to the target number. Similar ratio dependence has been observed by Beran and colleagues (2006) when tested adult humans employing articulatory suppression. Adults required to repeat the alphabet aloud were less accurate and their performance was more dependent on the ratio of the sets to be compared in comparison with adults who were allowed to count.

All these findings taken together show that even adult humans with knowledge of symbolic mathematics, represent quantities through the ANS when they have no access to verbal count.

Developmental studies provide far more evidence of an ANS. Newborns discriminate a 1:3 ratio (4 vs. 12) but not a 1:2 ratio (4 vs. 8) (Izard et al., 2009); six-month-old infants proved be able to discriminate arrays of dots that differ by a ratio of 1:2 (4 vs. 8, 8 vs. 16, 16 vs. 32) but not 2:3 (8 vs. 12, 16 vs. 24) (Xu & Spelke, 2000; Xu, 2003; Xu et al., 2005), whereas 10-month-old infants can discriminate numerosities with a 2:3 (8 vs. 12) but not a 4:5 (8 vs. 10) ratio (Xu & Arriga, 2007). The resolution of this system continues to increase throughout childhood, with 6-year-olds being able to discriminate a 5:6 ratio and adults a ratio of 9:10 (Halberda et al., 2008). On the whole, these studies indicate that this capacity is normally present at birth and increases in precision during development.

Comparative studies have reported ratio dependence also in animals. Cantlon and Brannon (2007b) used a non-verbal arithmetic task to compare adult humans and rhesus monkeys' ability to perform mental addition. Both monkeys and college students were presented with two dot arrays on a screen that disappeared after 500 ms to prevent humans from counting the items in each array. Subsequently it was given the choice between a set corresponding to the sum of dots previously seen and a distractor set containing a different numerosity. Despite accuracy was greater in college students,

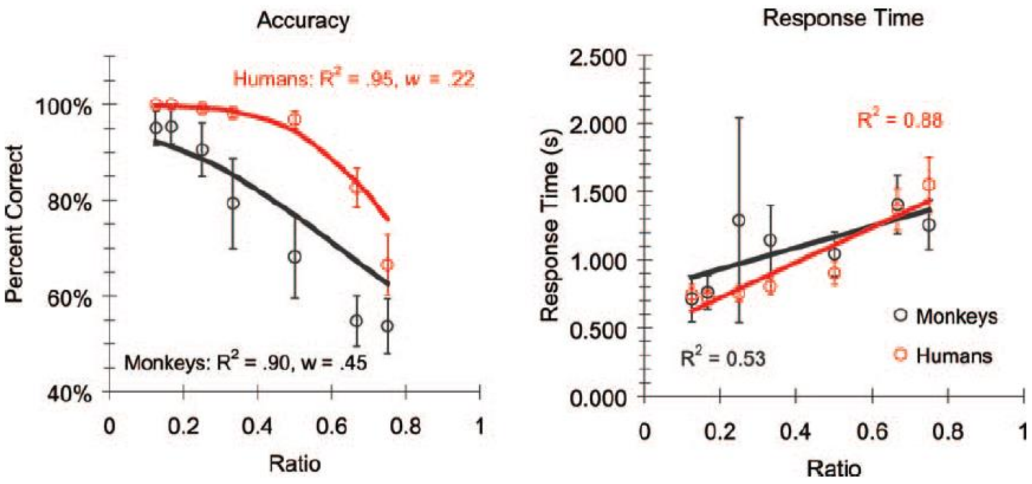


Fig. 7: *Accuracy and reaction time are similarly affected by numerical ratios in the large and small number range both in rhesus monkeys and adult humans (Cantlon & Brannon, 2007b).*

monkeys and humans' performance was qualitatively similar and was constrained by the numerical ratio between the correct sum and the distractor value. This similarity not only yields evidence of monkeys' ability to mentally sum quantities but also points toward a continuity in the cognitive processes underlying approximate quantity discrimination across the species (Fig. 7).

Similarly, when dogs (*Canis lupus familiaris*)were presented with two plates containing a different number of food treats (from 1 to 5 pieces), they chose the larger quantity of food but their performance decreased with increasing ratio: dogs were more accurate in 1 vs. 4 discrimination than in 3 vs. 4 (Ward & Smuts, 2007). Baker and colleagues used the same procedure to test coyotes (*Canis latrans*) (2011) and re-test dogs (2012) finding similar results to those previously obtained by Ward and Smuts (2007): numerical ratio was the best predictor of the performance.

Al Aïn and colleagues (2009) studied African grey parrots' (*Psittacus erithacus*) ability to discriminate discrete (seed) and continuous (liquid) amount of food. Birds were tested in their ability to select the larger number of food items (from 1 to 5) or the larger volume of a food substance (from 0.2 to 1.0 ml). Accuracy was correlated with the numerical ratio in both experiments with worst performance when the ratio was large.

These findings suggest that the ANS underlies quantity judgements in animals and support the hypothesis that we share with animals similar mechanisms for representing approximate quantities with a long evolutionary history.

Despite a wealth of studies supports the existence of an unique approximate system for the whole numerical range, some authors claim the existence of a second core system, uniquely involved to represent small quantities.

When adult humans are required to enumerate the items in a dot array, they are precise and fast for numerosities up to 4 objects but their accuracy decreases and the reaction time increases beyond this range (Mandler & Shebo, 1982; Pylyshyn & Storm, 1988, Trick & Pylyshyn, 1994). The process underlying the fast and accurate enumeration of small numerosities without real counting is known as subitizing (Kaufman et al., 1949, Jevons, 1871; Revkin et al., 2008) and it has been proposed to be based on an evolutionarily ancient system, known as the object tracking system (OTS).

The primary purpose of this system is supposed to be not quantitative but rather, its original function seems to be keeping track in parallel of objects moving in the space even if underwent brief periods of occlusion (Trick & Pylyshyn, 1994; Scholl & Pylyshyn, 1999, Assad & Maunsell, 1995). This mechanism would have been particularly useful in social species, for example, to track individual members of a moving group or, to track the presence of possible competitors or prey. This system would have been co-opted for numerical discrimination since object representations indirectly contain information about the number of items in the array, due to the one-to-one correspondence between each real item and its mental representation stored in the working memory. The OTS was initially described to explain adults' ability to track small sets of items (Kahneman et al., 1992; Pylyshyn & Storm, 1988; Scholl, 2001), but researches increasingly suggest that it may also underlie small quantity discrimination in infants (Uller, 1999, Feigenson et al., 2002a, Spelke & Kinzler, 2007, Leslie et al., 1998).

As opposed to the ANS, the OTS is accurate and shows no ratio effect (i.e., performance is similar in 1 vs. 4 and 3 vs. 4 comparison), but it is characterized by a set-size signature, which reflects the inherent limit of short term memory to store/represent simultaneously no more than 3-4 objects (Trick & Pylyshyn, 1994; Scholl & Pylyshyn, 1999).

Evidence of this system comes from studies reporting a set-size limit of 3 items in infants' quantity discrimination ability.

When 10-to 12-month-old infants were given the choice between two sets of crackers they approached the container with the larger quantity when the choice was between 1 vs. 2 and 2 vs. 3 crackers but failed when the numerical comparisons were 3 vs. 4, 2 vs. 4 and 3 vs. 6. These data suggest that infants relied on the OTS rather than on the ANS which would have predicted a successful choice in both 2 vs. 4 and 3 vs. 6 due to the same numerical ratio of 1 vs. 2 (0.5). Furthermore infants' performance dropped when one set contained more than 3 items in accordance with the set-size signature of object representations. However, in control tests, the authors observed that infants' choice was based on the total area rather than on number and suggested that infants represented each item as a distinct individual and then summed their area to represent the total amount of food. One possible explanation is that each object-representation conveys information about the real object they represent (such as, area, colour): in this way infants can compare the physical properties of the representations to make their choice (Feigenson et al., 2002a).

In a further study, Feigenson and Carey (2003) provided evidence of infants' ability to compare sets using number (via one-to-one correspondence) through a manual research task. Twelve- to 14-month-old infants could see the experimenter placing one ball into a box and were allowed to retrieve it. Then they observed two balls being placed inside the box but, one of them was removed from the back of the box out of infants' view. Authors compared the amount of time spent to search for the ball between the condition in which infants saw 1 ball hidden and expected to retrieve 1 and the condition in which they expected 2 balls but were allowed to retrieve only one. When subjects retrieved the single expected ball in the first condition, they stopped searching whereas they searched longer for the second ball when they expected 2. A similar behavior was recorded when they could see 2 balls hidden and could retrieve both balls compared with the condition where they expected 3 balls but could take only 2. Conversely, when infants watched 4 balls but 2 were removed, they stopped searching after retrieving the remaining 2 balls. Infants proved be able to represent exactly arrays with 1, 2 and 3 objects but not sets with 4 objects. Infants failed even when an easier numerical comparison was presented (1 vs. 4) both sequentially (manual research paradigm) and simultaneously (food choice task) thus confirming the striking limit of 3 units in infants (Feigenson & Carey, 2005).

Furthermore other experimental paradigms reported that small quantity discrimination is independent from the ratio in accordance with the hypothesis of a second mechanism different from the ANS. Six-month-old infants proved be able to discriminate a 0.67 ratio in the small number range (2 vs. 3: Kobayashi et al., 2005; Jordan & Brannon; 2006) but not in the large number range (8 vs. 12: Xu & Spelke, 2000; Lipton & Spelke, 2003).

Other evidence of a second core system comes from studies showing that infants consistently fail to compare small (≤4) and large sets (≥4).

Infants do not pick the larger number of crackers when the choice is between 1 vs. 4, 2 vs. 4 and 3 vs. 6 (Feigenson et al., 2002a, Feigenson & Carey, 2005), and they stop searching for balls after retrieving 1 or 2 ones from a box even though they have seen 4 balls being hidden (Feigenson & Carey 2003, 2005). Six-month-old infants discriminate 4 vs. 8 jumps or dots when continuous quantities are controlled but not 2 vs. 4

(respectively: Wood & Spelke, 2005, Xu, 2003) and 10- to 12-month-old infants prefer the larger amount of food items (Cheerios) when quantities are both small (1 vs. 2) or large (4 vs. 8) but not when one is small and the other one is large (2 vs. 4 and even 2 vs. 8) (vanMarle, 2013). If they had used the ANS they should have succeeded in all comparisons due to the easy ratio (0:5 or even 0.25). Infants' failure in comparing sets across the boundary between the small and large numbers has been explained in terms of incompatibility between the two systems (Xu, 2003, Feigenson et al., 2002a, vanMarle, 2013). It is possible that infants represent small number through the OTS and large number through the ANS creating two different kinds of representations impossible to be compared..

Further evidence supporting the two-system hypothesis is provided by Revkin and colleagues (2008). Adult humans were required to name the numerosity of dot arrays ranging from 1 to 8 dots and from 10 to 80 dots with the assumption that if only a single system is engaged for both small and large quantities, performance should be similarly affected by ratio along all numerical contrasts, irrespective of the range (≤ 4 or ≥ 4). Results showed that subjects were faster and more accurate in the subitizing range (1-4) and that discrimination was independent from the ratio. Conversely accuracy decreased and reaction time increased in the comparison 10 through 80, revealing also a ratio effect, in line with the idea of two separate mechanisms involved in discrimination of small and large sets (Fig. 8).

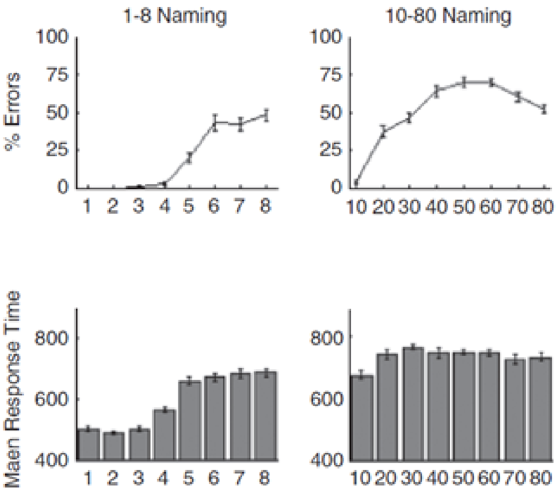


Fig. 8: *Adult humans were more accurate and faster over numerosities 1-4 than over numerosities 10-80, in contrast with the hypothesis of a single system to process both small and large quantities (Revkin et al., 2008)*

Apart from behavioral researches, which leave open the question about the existence of one or two systems, recently, psychophysiological and neuroimaging studies have provided additional data in favour of the hypothesis of two distinct systems. Hyde and Spelke (2009) recorded the event-related potentials when participants were presented with arrays of small (1-3) and large number (8-24) dots. They observed an early-evoked component (N1), modulated by absolute number with small number arrays and a later component (P2p), modulated by the numerical ratio between large dot arrays, but not small, numbers

In addition, Vetter and colleagues (2011) studied the neural correlates of visual enumeration using fMRI and showed that manipulating attentional load modulated the neural signal specifically in the subitizing range (up to 3 items), and moreover, in the right temporo-parietal junction, rather than in the IPS, the locus for larger number estimation and discrimination (e.g. in Piazza et al., 2003). These results bolster the hypothesis that adult humans spontaneously process both small and large quantities but through different systems.

Comparative studies add further evidence of a precise mechanism involved in small quantity representation in animals.

As previously reported, salamanders preferred the larger number of fruit flies when the choice was between 1 vs. 2 (0.50) and 2 vs. 3 (0.67) but not 3 vs. 4 prey (0.75), showing an upper limit of 3 units in small quantity representations similar to that of infants (Uller et. al., 2003). In addition they failed in 4 vs. 6 discrimination even though the same ratio as 2 vs. 3 (0.67). If numerical ratio is the best predictor of the performance, we should expect the same discrimination ability in both small and large number range. On the contrary, performance was affected by ratio, in large quantity discrimination of prey: salamander preferred 16 crickets to 8 but not 12 to 8 (Krusche et al., 2010). The set-size limit in small numerosities and the ratio dependence in large numerosities support the existence of two distinct mechanisms.

Other studies have found the typical hallmark of the object tracking system even if the limit of the representational system is not 3 items like in infants, but 4 items like in adults.

Rhesus monkeys presented with apple slices sequentially hidden in two boxes picked the larger quantity in 1 vs. 2, 2 vs. 3 and 3 vs. 4 comparisons, but not in 4 vs. 5 and 4 vs. 6 ones (Hauser et al., 2000). Similarly, New Zealand robins successfully discriminated 1 vs. 2, 2 vs. 3, and 3 vs. 4 mealworms, but they required at least a 1:2 ratio (4 vs. 8) to discriminate larger numerosities (≥ 4) (Hunt et al., 2008) in contrast with the existence of an unique system based on the ratio.

In a semi-naturalistic study on free-ranging dogs, Bonanni and colleagues (2011) observed that the decision to attack another group was based on the numerical ratio between one's own group and the opposing one when packs contained more than four dogs. Conversely, when both groups were small (≤ 4 individuals), dogs approached the opponent pack with the same probability when they outnumbered the opponents by a 1:2, 2:3, or 3:4 ratio, suggesting that they were able to precisely assess the group size irrespective of the ratio.

Despite multiple findings in favour of the two-system hypothesis data are not consistent and there is an on-going debate about the mechanism underpinning small and large number representations. In short, while the existence of the ANS is generally accepted, researchers tend to disagree as to whether a distinct precise system operates within 3-4 units.

In contrast to the existence of separate cognitive mechanisms, empirical evidence supports the existence of a single supramodal and domain-independent core system of number representation in both humans and non-human animals to account for both small and large discriminations (Izard et al., 2009, Jordan et al., 2005, Gallistel & Gelman, 1992).

For instance, similar ratio limits have been observed in 6- to 9-month-old infants' ability to discriminate large quantities with both visual-spatial arrays and auditory-temporal sequences (Xu & Spelke, 2000; Lipton & Spelke, 2003). Interestingly, the ANS seems to be also engaged to discriminate small quantities in the auditory domain.

vanMarle and Wynn (2006) investigated 6-month-old infants' ability to discriminate between durations with a 1:2 and 2:3 ratio. Infants were habituated to audio-visual sequences that lasted either 2 or 4 seconds (or 0.5 vs. 1 s. in a second experiment). In test phase both the events were alternated and infants looked longer at the novel one. However, when tested with duration differing by a 2:3 ratio (3 vs. 4.5 seconds or 0.67 vs. 1 second), they did not discriminate between the events. Likewise, 7-month-old infants discriminated sequences of 2 vs. 4 tones but not 2 vs.3 tones, matching the results obtained in the duration task (vanMarle & Wynn, 2009).

Furthermore Cordes and Brannon (2009) showed that 7-month-old infants were able to discriminate small from large arrays of dots when the ratio was at least 1:4 (1 vs. 4 and 2 vs. 8) providing data at odds with previous findings.

On the whole, the same ratio limits are consistent across modalities in infants: 6-month-old infants are able to discriminate a 1:2 ratio but not a 2:3 one, 9- to10-month-old infants discriminate a 2:3 ratio but not a 4:5 ratio (single item area: Brannon et al., 2006; auditory stimuli: Lipton & Spelke, 2003; van Marle & Wynn, 2006, 2009; number of multiple items: Xu & Spelke, 2000; Xu et al., 2005; Xu & Arriga, 2007; actions: Wood & Spelke, 2005). In addition a 1:4 ratio is required to discriminate the cumulative area of multiple items (Cordes & Brannon, 2008), to compare small and large quantities (Cordes & Brannon, 2009) and to discriminate between substances (vanMarle & Wynn, 2011).

Nonetheless, Tokita and colleagues (2013) disagree with the idea that humans and non-human animals share a single modality-independent cognitive mechanism because they found clear difference in adults' performance across modalities: subjects were more accurate with auditory sequences than with visual arrays and performance of cross-modal comparisons laid between the performance of visual and auditory trials.

Other authors suggest the existence of multiple core number systems engaged to solve a limited set of problems and that operate independently from each other (Feigenson et al., 2004; Spelke, 2000).

When infants are presented with sets of food items (crackers) smaller than 4 units, they discriminate quantities via OTS but their representation is based on the summation of continuous properties rather than on number (Feigenson et al., 2002a). However in a manual research task, infants exactly represent up to 3 objects even when continuous variables are controlled suggesting that they rely only on numerical information (Feigenson & Carey, 2003, 2005). The different salience of number may be due to the kind of stimuli and task used. When the goal is obtaining a certain number of objects, the choice on the basis of their continuous extent instead of the number is not useful to solve the task. On the contrary, when the stimulus is food, it is probable that infants respond to perceptual features (i.e., surface area) since they are more informative of the total amount of food in order to maximize the food intake. As a consequence it seems that infants can make different computations over object representations depending on the task. This is supported by experiments in which infants were tested using the habituation procedure with three-dimensional objects. When the sets to be discriminated were composed by identical objects, infants detected changes in continuous extent but not in number, conversely, when heterogeneous stimuli were presented, they showed be able to represent the number of items in the array (Feigenson, 2005).

Not only the type of stimulus but also the way in which it is presented influences infants' performance. VanMarle and Wynn (2011) showed that 10-month-old infants discriminated food presented as discrete entities (1 vs. 2 crackers or 5 vs. 10 cheerios sequentially hidden) when the numerical ratio was 1:2, but they required a 1:4 ratio to discriminate food presented as substances (two portions of cheerios poured in cups to make them as substance-like as possible). In addition, in the substance-like condition, infants failed when density or perimeter were equated in contradiction with previous data that showed a robust ability to represent discrete quantities when continuous variables were controlled (Lipton & Spelke, 2003; Wood & Spelke, 2005; Xu & Spelke, 2000), suggesting that infants need multiple cues when comparing substances.

Furthermore it is important to consider that the OTS is thought to be a mechanism for tracking multiple visible objects; thus when sounds are represented, it is unlikely that this system is activated since there are no objects to be represented. Data seem to confirm this assumption. Six-month-old infants successfully discriminate small sequence of audiovisual events with a 1:2 ratio but not with a 2:3 ratio as expected by the hypothesis that in absence of visual stimuli, the approximate system and not the object system is involved (vanMarle & Wynn, 2006;2009). Conversely, Lipton and Spelke (2004) observed that 9-month-old infants failed in 2 vs. 3 sounds discrimination, a numerical contrast expected to be solved at that age if the ANS has been involved.

Mixed results are also reported in comparative studies. For example, when Beran (2007) tested rhesus macaques using a computerized version of the item-by-item procedure in which no food but red squares were hidden (from 1 to 10), monkeys' performance was affected by the numerical ratio throughout the whole numerical range and no set-size limit was detected in small quantities, in contrast with results provided by Hauser and colleagues (2000). Dogs and coyotes' preference for the larger quantity of food treats (1 to 5) is a function of the ratio between quantities (Ward & Smut, 2007;

Baker et al., 2011, 2012) whereas wolves choose 1 vs. 4 pieces with the same accuracy as 3 vs. 4 (Utrata et al., 2012).

In conclusion, results across studies are not entirely consistent, and it is unclear whether these differences are due to the task presented, the sensory modality involved, the context or if they are related to differences among the species investigated.

To date it is difficult to understand whether animals possess a single or multiple systems of numerical representation since very few studies have investigated numerical abilities in the same species across different contexts.

The same problem occurs in human literature, In particular it is difficult to design experimental paradigms suitable for testing at the same time newborns and infants since sensitivity at stimuli and the level of attention may change over development.

1.4. Development of pre-verbal numerical abilities

Developmental studies are fundamental to assess which cognitive skills a species is naturally endowed with since comparing performance at different ages may provide insights about the existence of common cognitive systems at work during development.

In particular in the domain of numbers, the study of numerical abilities in non-verbal creatures (pre-verbal infants and animals) is relevant to increase our comprehension of non-symbolic representation of number in absence of language. To date the literature on the ontogeny of numerical competence is almost entirely focused on human infants and provides evidence that infants' numerical abilities are continuous with those of adults (Hauser & Spelke, 2004).

1.4.1. Developmental studies in humans

As previously reported, several studies have described numerical abilities in infants.

Early studies on infants' numerical skills focused on the ability to discriminate small sets of figures using the habituation procedure. Infants were first presented with stimuli containing a fixed number of items and when they were habituated novel numerosities were shown in test phase. The procedure relied on a preference for novelty and the assumption was that if infants were able to detect the difference in number they would look longer at the novel numerosity.

Starkey and Cooper (1980) habituated 4- to 7-month-old infants with linear arrays of either 2 or 3 dots with different density and total line length in order to avoid discrimination by configuration. After habituation, infants were shown with either an array containing the familiar number of dots or an array with the novel numerosity. Infants looked longer at the new quantity, thus suggesting that they detected a change in number. However, when tested with 4 vs. 6 dots, no difference in their looking time was perceived. Subsequent studies obtained similar results. Antell and Keating (1983) replicated the experiment with dots in newborns whereas Strauss and Curtiss (1981) habituated 10- to 12 month-old infants to pictures of items (e. g., dogs, houses, etc.) that varied in number and found that subjects discriminated 2 vs. 3 as well as 3 vs. 4 items. Together, these pioneering studies suggested that infants are sensitive to number since they looked longer at the novel numerosity.

Wynn (1992) drew similar conclusions using the expectancy violation procedure. The method consisted in presenting the subjects with simple arithmetic problems (such as easy addition or subtraction) and then comparing the looking time between an unexpected and an expected numerical outcome. In this experiment, one toy was placed on a stage, a barrier was raised to hide the item and then the experimenter placed a second toy behind the screen. If infants were able to compute simple arithmetical operations it was expected they would look longer at an unexpected result (1 + 1 = 1) than at an expected one (1 + 1 = 2) (Fig. 9). Five-month-old infants looked longer at the incorrect output both in an addition and in a subtraction task and the author concluded that infants were responding to the change in number.

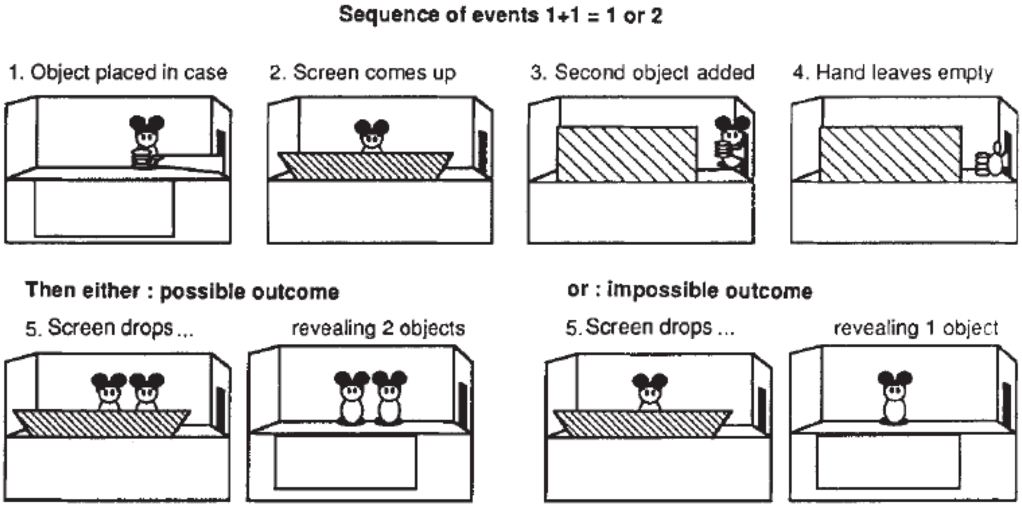


Fig. 9: *Expectancy violation procedure adopted to study whether infants can represent numerical information. Infants were presented with simple arithmetic problems and then the looking time between an unexpected and an expected numerical outcome was compared (Wynn, 1992).*

However all the studies reported so far, did not properly controlled for continuous quantities and number was always correlated with perceptual features, such as, contour length or area. As a consequence, although infants proved be able to discriminate small quantities, the nature of this discrimination was unclear.

Clearfield and Mix (1999) observed that 6- to 8-month-old infants dishabituated only to change in continuous quantities but not in number and suggested that the relevance of perceptual cues had been underestimated in previous researches.

This result was confirmed by Feigenson and colleagues (2002b), who adopted the habituation/dishabituation procedure using three-dimensional objects to further investigate infants’ sensitivity to continuous extents when presented with small sets of items. When homogeneous stimuli were controlled for surface area and other continuous variables (i.e., total volume, contour length, brightness and total spatial envelope), 6- to 7-month-old infants did not respond to change in number, in agreement with Clearfield and Mix (1999). Conversely, when infants were habituated and tested with heterogeneous objects differing for colour, pattern and texture, they were able to compute number (Feigenson, 2005). One possible explication for the different representations might be that dissimilar items are easier to be remembered. It is possible that infants are capable to represent two objects for two identical items but their similarity can make it difficult to recall them as different items. Alternatively, infants may fail to create a new object-representation for an item already represented when objects have the same properties. On the contrary, it seems to be easier to maintain items as distinct individuals when they differ for some details and it facilitates the discrimination based on number. In conclusion, when objects are similar, infants represent continuous variables, but when objects are different, infants compute number.

The habituation procedure has been widely used in literature not only with visual arrays but also with other kinds of stimuli such as, series of events, sequences of tones and durations.

For instance, infants habituated to sequences of either 2 or 3 puppet jumps, looked longer at the sequence with the new number of jumps in test phase. However, although the sequences were controlled for total duration and rate, the total amount of movement was greater in the three-jump series and it was not possible to exclude that infants used the motion as a cue rather than the number of jumps (Wynn, 1996). van Marle and Wynn (2006, 2009) used the habituation technique to investigate infants' ability to discriminate auditory stimuli controlled for non–numerical properties to ensure they used numerical information. In both cases 6- to 7-month-old infants' performance followed Weber's Law since they succeeded in the 1:2 ratio but failed in the 2:3 one.

However data on infants' representation of small quantities are inconsistent since different procedure yields to different conclusions.

In a food choice task, 10- to 12 month-old infants' discriminated between 1 vs. 2 and 2 vs. 3 crackers but in control test their choice was determined by total surface area or total volume, accordingly to the predominant idea that infants are able to compute continuous variables but not number (Feigenson et al., 2002a)

Conversely, Feigenson and Carey (2003) showed that 12- to 14-month-old infants tested in a manual research task were able to represent the individual items up to 3 objects even when the authors controlled for the continuous variables. For instance the infants could see two small objects being hidden in a box but could retrieve only one big. If their searching behavior was based on the total volume to be retrieved, they should expect the box empty and should stop searching. On the contrary, if they were relying on number they should continue searching, expecting the box to contain more items. Infants continued to search after retrieving the big object thus showing that they noticed the difference in number and did not rely on volume.

The discrimination in the small number range has been investigated also using cross-modal comparisons to assess whether infants are able to match the number of items they could see with the number of sounds they could hear. In a study by Kobayashi and colleagues (2005), six-month-old infants were initially familiarized with two and three toys dropping and emitting a tone when they impacted the surface. In test trials subjects could just hear two or three tones but the toys' motion was hidden by a barrier occluding infants' view. When the barrier was removed infants could see two or three toys on the stage and looked longer at the unexpected outcome (i.e., they heard two tones but three toys were on the stage). The authors interpreted the result as evidence of infants' ability to recognize the numerical equivalence across modalities.

In a further study, Jordan and Brannon (2006) reported that infants possess the ability to match the number of women seen on a screen (visual stimuli) with the number of voices heard (auditory stimuli) even when potential confounding factors such as the duration and the rate were controlled for. In their experiment, 7-month-old infants looked longer at the display showing the number of women (2 or 3) that numerically matched the number of voices (2 or 3) saying the word "look" in comparison with the non-matching display.

To date, these findings provide evidence of infants' ability to discriminate small quantities but it is still debated which information (discrete or continuous) they use.

Conversely, data collected on large quantity discrimination are more consistent across modalities and reveal that infants' representation of large numerosities is approximate and affected by ratio, but also that the accuracy increases over development until adulthood, supporting the idea of a continuity in numerical abilities. Furthermore, the convergence of the results in studies using different procedures provides unequivocal evidence of infants' sensitivity to number in the large number range (see Section 1.3). Infants can discriminate between 8 and 16 items with both visual and auditory stimuli strictly controlled for continuous variables (Xu & Spelke, 2000; Lipton & Spelke, 2003).

Interestingly, Izard and colleagues (2009) tested 2-day-old newborns in a numerical cross-modal matching task and yielded evidence of approximate number skills even early in the development. Newborns were familiarized with sequences of either 4 or 12 syllables (1:3 ratio) and then were presented with arrays of two-dimensional items containing the same or a different number of figures. Subjects looked longer at the display numerically matching the sequence of syllables and the data was confirmed by a second group tested with 6 vs. 18 (1:3 ratio). However newborns failed in the 4 vs. 8 contrast, thus revealing the typical ratio limit signature of the ANS observed in older infants and adults.

The discrepancy between the results obtained in the small and in the large number range is still unexplained. It is possible that when infants face with small sets, they focus their attention on the individual objects rather than on the number and hence are unable to perceive the cardinal value (Xu et al., 2005), but further studies are required to shed light on the difference between the two systems.

On the whole, these data show that infants are endowed with numerical systems early in the development but the inconsistency of the results highlights the limits of studying the ontogeny of numerical cognition in our species. For ethical and practical reasons it is difficult to manipulate infants' experience during development and it hampers the comprehension of the relative salience of maturation and experience. Furthermore different procedures are used at different ages and it is difficult to make a fine comparison of collected data. With respect to this topic, animal models may represent an useful tool, in particular those species with a short life–span, because it is possible to study the developmental trajectories of cognitive abilities through newborns, juveniles and adults in a relatively short amount of time.

1.4.2. Developmental studies in animals

Presently, longitudinal data are available only for human infants and little is known about how numerical skills unfold over development in animals.

Domestic chicks (G*allus gallus*) in particular have become a preeminent model, given the possibility they present to controlled-rearing and testing conditions.

Rugani and colleagues (2007) initially investigated whether chicks possess ordinal abilities, namely, if they were able to identify a certain location/object in a series of identical ones on the basis of its position. To address the question, 5 day- old chicks were trained to peck either at the 3rd, 4th, or 6th hole in a series of 10 identical aligned holes spaced 1 cm from each other. The chicks successfully selected the correct location above chance level. However, since animal could have used geometrical-spatial cues to solve the task, the authors carried out a series of experiments to control for potential non-numerical cues. In one experiments, chicks were initially trained to peck at the 4th location but, in test phase, the series of holes was rotated by 90° and was horizontally positioned instead of being aligned with the starting point. In the second experiment, the authors manipulated the distance between the holes to assess if chicks chose the correct one on the basis of the absolute distance from the starting point or if they relied on the ordinal position of the correct hole. To further avoid the use of potential geometrical cues, in the last experiment chicks were tested in a circular arena and were trained to retrieve food from the 3rd jar in series of identical ones and the position of the jars was changed from one trial to the subsequent one. Chicks successfully chose the location they were trained to in all conditions providing evidence that ordinal abilities are inborn in this species. It was also observed that, in absence of asymmetrical cues, the subjects spontaneously preferred to approach the correct location starting from the left end of the series and not from the right. Ordinal competency and preference location starting from the left was later confirmed by Rugani and colleagues (2010a), who advanced the suggestive idea of a mental number line in chicks' brain, even though other explications were possible, such as a bias in the allocation of their attention when choosing. Based on this evidence, the capacity to take into account ordinal information seems to be present at birth, and might have been selected due to its potential adaptive value. It is possible to imagine ecological contexts in which displaying such a skill might be useful from the first days of life, for instance, when searching for a source of food ('after three rocks') or a shelter ('the third cave') for predator avoidance.

Other studies show that the domestic chick is able to represent also the cardinality (the number of items in an array) of small sets even when continuous variables are equated to avoid subjects from using them.

Chicks were trained to discriminate between arrays containing different number of dots or squares in order to get a food reward (Rugani et al., 2008). They successfully learned the discrimination in 1 vs. 2 and 2 vs. 3 comparisons, even when stimuli were controlled for contour length and overall surface but they performed at chance level in 3 vs. 4, 4 vs. 5 and 4 vs. 6. The results shows that chicks have a set-size limit of 3 units in the small number range, in line with data in infants literature, and that are able to represent numerical information in small quantity discrimination.

Simple arithmetic seems also to be processed by 5-day-old chicks (Rugani et al., 2009). Chicks were initially reared with five identical objects that served as imprinting objects. During test phase, subjects could see two sets of objects; one composed by 3 of the 5 imprinting objects and the other made of the remaining 2, being hidden sequentially or simultaneously behind one of two screens. After the disappearance of both sets the chicks spontaneously preferred to join the larger number of imprinting objects, probably because a larger group of them represents a sort of 'super-stimulus' (Rugani et al., 2010b). Interestingly, the chicks inspected the screen occluding the larger set even when continuous quantities were controlled, proving that they were able to keep track of single items relying on numerical information alone. In a subsequent experiment, after the initial disappearance of both sets (3 vs. 2), the researchers transferred some of the objects, one by one, from one screen to the other in full view of the subjects before releasing them into the arena. The chicks again selected the group containing the larger number of objects. In another test (4 vs. 1), the chicks were required to choose against the potential directional cue provided by the final visible displacement (indeed, in the 3[-1] vs. 2[+1], the chicks could have simply followed the last moving objects) by presenting the following comparison: 4(-1) vs. 1(+1). Even in this case, the chicks spontaneously chose the screen hiding the larger number of objects, irrespective of the cue provided by the final displacement thus showing proto-arithmetic capacities.

In a subsequent study (Rugani et al., 2011) when larger sets (5 vs. 10 and 6 vs. 9) were sequentially hidden behind a screen, the subjects chose the larger number of items only when both number and continuous quantities were simultaneously available; hence chicks seem to be sensitive to continuous extent and not to numerical information in large quantity discrimination, in contrast not only with previous results on the same species (Rugani et al., 2008, 2009) but also with data collected in infants (Xu & Spelke, 2000, Izard et al., 2009).

However, it seems that the ability to rely on number depends on the characteristics of the objects used to rear the chicks (Rugani et al., 2010b). In one condition, chicks were imprinted on either 1 or 3 yellow plastic balls. In the absolute discrimination test phase, subjects were presented with two arrays: one made of 1 imprinting objects and the other made of 3 imprinting objects. In the relative discrimination test phase, instead, chicks were presented with two sets composed by 4 plastic balls each, but one set contained only one imprinting objects and three similar balls but differing in colour whereas the other set contained three imprinting objects and a single different ball. Chicks chose the larger set, irrespective of the number of balls they were reared with, both in the absolute and in the relative discrimination task. However, when they were reared with objects differing in shape, size and colour and then were tested with sets containing other new and different objects, the subjects approached the array containing the same number of items they were imprinted on, even if the stimuli were completely novel and were equalized for volume and surface. This data aligns with those collected in human infants: infants represent number when they discriminate between heterogeneous stimuli but rely on continuous variables in presence of homogeneous items (Feigenson, 2005).

On the whole, these results largely parallel those obtained in human infants literature providing evidence that young chicks, and not only human infants, are endowed with numerical abilities early in development. Chicks, such as infants, exhibit the same 3 item-size hallmark of the OTS, compute continuous extents when tested with homogeneous items but number when tested with heterogeneous stimuli and show proto-arithmetic abilities.

However, despite performance are strikingly similar, chicks represent small arrays by using numerical information only but not sets larger than 3 items, a pattern of data that does not entirely align with infants (Clearfiled & Mix, 1999; Xu & Spelke, 2000; Izard et al., 2009). It is possible that human infants share with domestic chicks early developing systems of pre-verbal numerical cognition which are similar but not exactly the same, due to the different performance in the large quantity discrimination. It would be interesting to assess whether this numerical abilities change over development untill adult hen showing a pattern similar to the one observed in humans.

1.5. Numerical abilities in fish

Being able to process quantitative information provide multiple benefits to fish too. For instance, joining larger shoals allow fish to find food faster (Pithcer et al., 1982), to have hydrodynamic advantages (Barber & Folstad, 2000) and to decrease the risk to be spotted by predators (Foster & Treherne, 1978; Landeau & Terrborgh, 1986). For these reasons it is possible that selective pressure have favoured numerical abilities in fish. However, while there is a well-established tradition of studying numerical cognition in mammals and birds, researches have only recently focused on numerical abilities in fish, an animal model in behavioral and cognitive studies (Brown & Laland, 2003).

Agrillo and colleagues (2008a) exploited the spontaneous tendency in social species to re-join conspecifics when placed in an unfamiliar environment (Hager & Helfman, 1991; Pritchard et al. 2001; Buckingham et al., 2007) to assess numerical competence in fish. Female mosquitofish (*Gambusia holbrooki*) were singly inserted in an empty tank and two groups of conspecifics differing in number were presented at the two ends (Fig. 10). Fish spent significantly more time close to the larger shoals when the two groups differed by one unit (1 vs. 2, 2 vs. 3, and 3 vs. 4), while no choice was observed when groups larger than 4 fish were presented (4 vs. 5, 5 vs. 6, 6 vs. 7 e 7 vs. 8). Larger numerosities were discriminated only when numerical ratio between the two groups was at least 0.5 (4 vs. 8, 8 vs. 16 e 4 vs. 10).

Recently, Gòmez-Laplaza & Gerlai, (2011a, b) observed a similar pattern of choice in the small and large number range in angelfish (*Pterophyllum scalare*). Fish discriminated 1 vs. 2 (0.50) and 2 vs. 3 (0.67) conspecifics while their capacity to discriminate between large numbers was limited up to a ratio of 0.56 (5 vs. 9).

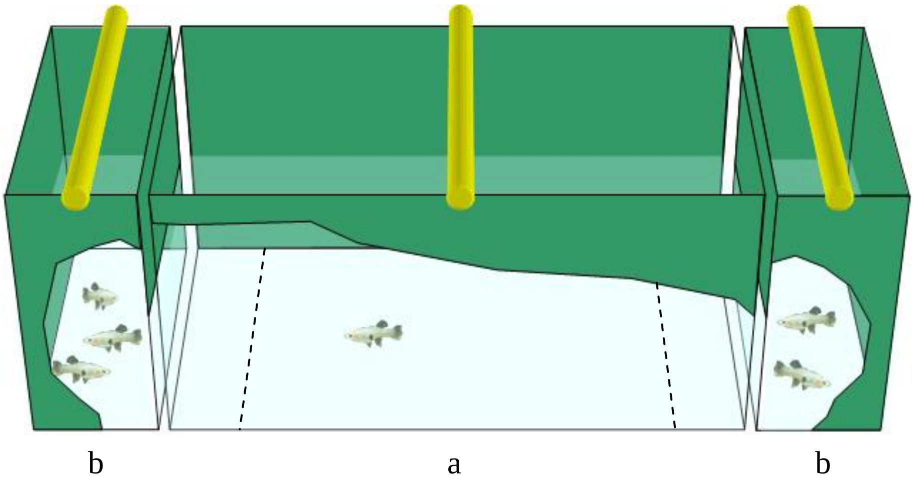


Fig. 10: *Schematic representation of the experimental apparatus used in spontaneous shoal choice. Subject was inserted in the central tank, subject tank (a) and two groups of conspecifics differing in numerosity were inserted at the two ends, stimulus tanks (b)(Agrillo et al., 2008).*

The different ratio sensitivity for small and large numbers suggests the existence of two distinct numerical system in fish: one precise and accurate for small numerosities (≤ 4) and one dependent on numerical ratio when large quantities are involved (≥ 4).

However, in the studies mentioned above, number is always correlated with continuous variables and it is difficult to assess the mechanism adopted by fish to select the larger shoal.

For example, when total surface area occupied by stimulus fish was equated, using smaller individuals in the large shoal and larger individuals in the small one, mosquitofish did not select any group, both in 2 v. 3 and in 4 vs. 8 comparisons, suggesting the relevance of cumulative surface area in shoal choices (Agrillo et al., 2008a). Similarly Gòmez-Laplaza and Gerlai (2013) found that angelfish were unable to discriminate between large (5 vs. 10) and small (2 vs. 3) shoals when the surface area of the contrasted shoals was equated.

The quantity of movement of stimulus fish represents another factor that might indirectly reveal the numerosity of a shoal. Most fish can live in a wide range of temperatures and their activity is directly influenced by water temperature. An increase in water temperature can determine an increased activity of the individuals. For instance, Pritchard and colleagues (2001) observed that zebrafish generally preferred the larger shoal in a 4 vs. 2 discrimination when the two stimulus shoals were in water of the same temperature. However, this preference decreased if the activity of the larger shoal was reduced by using colder water in their tank. The same technique was adopted with mosquitofish by Agrillo and colleagues (2008a), showing that the total movement of stimulus fish influenced the choice for the larger group differently in presence of small (≤ 4) or large (≥ 4) shoals. When the authors controlled for the quantity of movement, keeping the water temperature in the small group tank at 29 ± 1 °C and in the large one at 19 ± 1 °C, mosquitofish selected the larger shoal only in 2 vs. 3 but not in 4 vs. 8 contrast, suggesting that total movement was an additional cue necessary in large quantity discrimination but not in the small one. Subsequently, similar results have been obtained in angelfish. Gòmez-Laplaza and Gerlai (2012) designed two experiments to control for the total movement: in one experiment they used the same procedure adopted with mosquitofish, whereas in the second one they kept the stimulus fish in restricted compartments in order to reduce their swimming activity. In the first condition, angelfish preferred the larger shoal over the small one in 2 vs. 3 but, similarly to mosquitofish, not when larger shoals were presented (5 vs. 10). However, using the second procedure, angelfish preferred the larger shoal both in 2 vs. 3 and in 5 vs. 10 numerical contrasts. It is difficult to hypothesize why different results have been obtained as a function of the technique adopted to control for the quantity of movement. This result, however, highlights the importance of using multiple experimental strategies to control for continuous quantities before drawing a firm conclusion about the influence on continuous quantities in numerical tasks.

One limit of spontaneous choice tasks is that subjects may attend to perceptual features rather than to number, since both kind of information can yield to comparable results. Also when researchers control for one continuous variable at a time (e.g. quantity of movement) the possibility exists that fish use other continuous variables (overall space) instead of number. To assess whether fish can use numerical information only, Agrillo and colleagues (2009) initially trained mosquitofish to discriminate between 2 and 3 geometrical figures not controlled for continuous quantities. Subjects were singly inserted in an unfamiliar environment provided with two doors, one associated with three figures and the other associated with two figures, placed at two opposite corners. To re-join social companions, subjects were required to discriminate between the two numerosities and select the door associated with the reinforced numerosity. When fish reached a learning criterion, they were admitted to test phase, where subjects were presented with 5 different sets of stimuli. In four of them, one perceptual cue was controlled at a time (total luminance, sum of perimeter, cumulative surface area and overall space occupied by figures) while in the last set stimuli where not controlled for continuous variables. Fish performance dropped to chance level when stimuli were matched for the cumulative surface area or for the overall space occupied by the arrays, indicating that these latter cues had been spontaneously used by the fish. In a second experiment, where all continuous quantities were controlled during the training, mosquitofish proved able to learn the discrimination, suggesting a pure use of numerical information like reported in mammals (Beran, 2007; Vonk & Beran, 2012) and birds (Rugani et al., 2008; Emmerton & Renner, 2006).

Although fish could be trained to use numerical information to discriminate between set of abstract stimuli it was not clear whether they were also able to spontaneously use number in a more ecologically context. To verify whether numerical information is naturally represented by female mosquitofish, Dadda and colleagues (2009) used a modified version of the item-by-item presentation of the stimuli. To this purpose, the apparatus previously used in the spontaneous shoal choice by Agrillo and colleagues (2008a) was modified: each stimulus fish was confined in separate compartments at the two bottoms of the subject tank and several opaque screens were inserted in the subject tank to prevent a global view of the groups and to allow the subjects to see no more than one stimulus fish at a time (Fig. 11). In this way, subjects were required to add the number of stimuli on one side, make the same operation on the other side and then compare the two numerosities in order to select the optimal shoal.

Results showed that mosquitofish spent more time near the larger shoal in 2 vs. 3 and in 4 vs. 8. Further control experiments for density of individuals and the overall space occupied by the stimulus shoals showed that no continuous quantities could be advocated to explain the choice of the larger shoal.

Agrillo and colleagues (2010) used the same procedure adopted to study small quantity discrimination (Agrillo et al., 2009), to investigate large quantity discrimination (4 vs. 8) in mosquitofish. Similarly to previous study, fish learned the discrimination when no control was made but failed when stimuli were controlled for cumulative surface area.

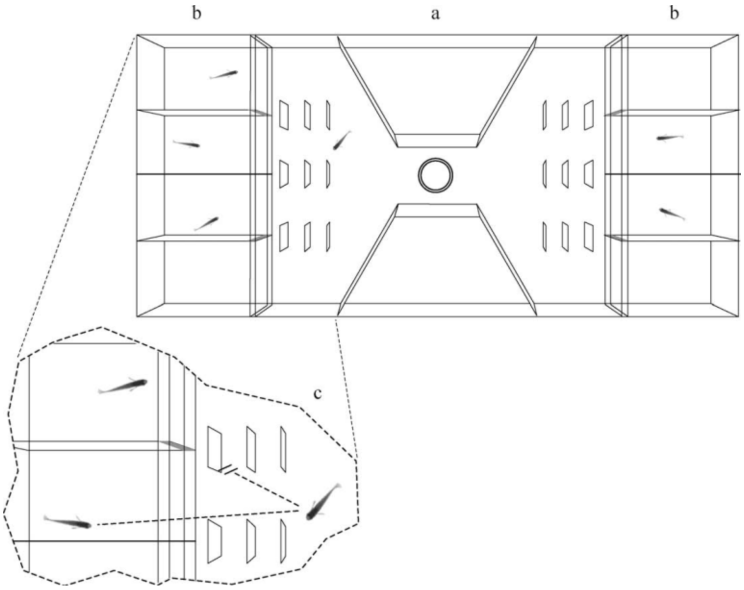


Fig. 11: *Schematic representation of the apparatus used in shoal choice task in 2 vs. 3 comparison. Subjects were singly inserted in a central sector (a). Each stimulus sector (b) was subdivided in identical compartments housing the stimulus fish. In each choice area artificial vertical screens were placed so that the subject could only see one stimulus fish at time from any position of its sector (c). (Dadda et al., 2009).*

However it is of interest that fish choice was not affected by the overall space occupied by the items whereas it was a relevant cue in the 2 vs. 3 discrimination. This difference, together with the different salience of the quantity of movement in the small and large number range (Agrillo et al., 2008a), might reflect the existence of two distinct numerical systems: one for small quantity and one for large quantity (Hauser et al., 2000; Feigenson et al., 2004). A similar conclusion was drawn by Agrillo and colleagues (2012) when tested guppies (*Poecilia reticulata*) in a spontaneous choice task. The same numerical ratios were used (0.25, 0.33, 0.50, 0.67, 0.75) for small (1 vs. 4, 1 vs. 3, 1 vs. 2, 2 vs. 3, and 3 vs. 4) and large (6 vs. 24, 6 vs. 18, 6 vs. 12, 6 vs. 9, 6 vs. 8) numerical contrasts. Fish discriminated up to 4 items with the same accuracy, whereas discrimination for large quantities was approximate and ratio dependent.

When Agrillo and colleagues (2010) controlled for all continuous quantities during training, mosquitofish proved able to learn the discrimination by using pure numerical information also when large quantities were presented. Furthermore, researchers carried out two more experiments to assess whether there was an upper limit in fish numerical discrimination and the influence of numerical ratio. Results showed that fish discriminated 100 vs. 200 as well as 4 vs. 8, revealing apparently no upper limit of numerical discrimination, while the numerical ratio had a clear effect on performance (accuracy decreased from 7 vs. 14 to 9 vs. 12). Finally, fish performance was compared with that of undergraduate students tested with the same stimuli while being prevented from using verbal counting. Data collected with humans replicated those observed in fish: although humans were obviously more accurate than fish, their pattern of accuracy was similar since both species showed a similar sensitivity to numerical ratio.

The ratio dependency in large number discrimination is a typical signature of ANS and it has been documented not only in humans but also in a wide range of animal species, suggesting that all vertebrates may share similar pre-verbal numerical systems. The nature of these systems is still debated: maybe it is the result of convergent evolution or it is inherited from a common ancestor.

Recently the ontogeny of numerical abilities has been investigated in fish too. Bisazza et al., (2010) used guppies as a model: this species is viviparous with relatively short lifespan and gives birth to fully developed offspring that are independent at birth and display a complex behavioral repertoire. In this sense, newborn guppies represent a suitable model for assessing the developmental trajectories of numerical abilities. Experiments were done using a small-scale version of the experimental apparatus adopted to study spontaneous quantity discrimination in adult fish (Agrillo et al., 2008a). Results showed that, at birth, guppies discriminated between shoals differing by one individual up to 4 units (1 vs. 2, 2 vs. 3, 3 vs. 4), but not when larger quantities where presented (4 vs. 5, 5 vs. 6). That is, the capacity of newborn guppies to discriminate between small numerosities is the same as that shown by adult individuals (Agrillo et al., 2012). At the same time, one-day-old fish were unable to find a difference in 4 vs. 8 (0.50 ratio) and 4 vs. 12 (0.33), suggesting that large quantity discrimination is absent or highly approximate at birth.

Because adult fish can easily discriminate between large shoals with at least a 0. 50 ratio (Agrillo et al., 2012), the development of large quantity discrimination (4 vs. 8) was investigated in a subsequent experiment. Guppies were tested at three different ages

(1, 20, or 40 day old) to assess the salience of maturation. Furthermore newborns were reared in two experimental conditions to evaluate the role of experience. In one condition fish were reared in pairs to allow a normal development of their social behavior but preventing them from seeing more than one fish at time. In the second one, fish were reared in group of 13 – 15 similar age fish with the possibility of seeing sub-groups of peers differing in numerosity. Fish from both experimental conditions were capable to discriminate 4 from 8 fish when they were 40 days old, but only subjects reared in groups were already able to choose the larger shoal at 20 days of age. This study showed that the ability to discriminate small sets is innate, whereas the large quantity system emerges only later as a result of both maturation and previous social experience. Overall, these results provide further evidence of the existence of two separate numerical systems. If the species is endowed with only one system of numerical representation, one would actually expect the same developmental trajectories for both small and large quantities: a condition not occurred in this study.

Finally, this study also included an experiment similar to that reported in adult fish using the item-by-item procedure (Dadda et al., 2009) to investigate whether newborn guppies were able to discriminate quantities by using numerical information only. Subjects could choose between two groups of conspecifics (2 vs. 3 and 4 vs. 8, presented respectively to newborns and young guppies) controlled for non-numerical cues, namely, the overall space occupied by the shoals, the density of the fish and the total surface from which stimulus fish were visible to the subjects (visibility space). Subjects joined the larger shoal in both numerical contrasts suggesting that, similarly to adults, juvenile guppies can process numerical information when tested in conditions that reduce the access to continuous quantities.

In conclusion, these results, in addition to the literature on chicks, show that some proto-counting abilities appear quite early, suggesting the existence of a 'number sense', that is inborn not only in primates (Feigenson et al., 2004; Izard et al., 2009), but also in other vertebrates.

2. AIM OF THE RESEARCH

In the last decade fish have become an useful model in numerical cognition studies. As previously reported, fish are able to select the larger shoal of conspecifics and can be trained to discriminate between groups of figures differing in numerosity both when allowed to use number and continuous quantities and when only number was available. Fish can also make a spontaneous use of numerical information with apparently the same effort required to discriminate continuous quantities. These abilities seem to be partially inborn as one-day old fish are already able to discriminate between small groups of peers.

Nonetheless some questions about numerical abilities in fish are still unanswered. For instance, it is unclear whether numerical systems are the same among different species, whether numerical acuity may be affected by different factors, such as cooperation among individuals and the presence of items in motion or whether newborn fish could be trained to discriminate between sets of items.

The aim of the present thesis was to fill this gap. In particular, the first part of the research deals with some of the open questions about numerical cognition in adult fish; the second part is focused on the ontogeny of numerical competence.

First of all, a novel training procedure was set up to investigate numerical abilities in fish. Recently it has been shown that fish can successfully be trained to discriminate between sets of two-dimensional figures, but the procedure previously used took long time and was stressful for fish. A new procedure was designed in which, at intervals, two stimuli representing different numerosities were introduced at opposite ends of the tank and food was delivered near the stimulus to be reinforced. Fish initially were trained on an easy numerical ratio (0.50). Once they reached the learning criterion, they were tested in non-reinforced probe trials for their ability to generalize to both small and large new numerosities. Time spent near positive stimulus in probe trials was taken as a measure of discrimination performance. To validate the method, two published experiments that used operant conditioning to investigate the mechanisms of numerical discrimination in mosquitofish were replicated (Agrillo et al., 2009, 2010). The replication of previous studies is useful to determine whether the results are simply an artifact of the methodology adopted or, conversely, they reflect the true cognitive abilities of the species. Since data indicated a complete overlap of the results obtained using the two different methods, the novel protocol was adopted in all the training experiments presented in this thesis.

The second study focused on a potential limit in numerical cognition research: the lack of cross-species studies using the same methodology. Adults, infants and non-human primates are thought to possess similar pre-verbal numerical systems, but there is considerable debate regarding whether all vertebrates share the same numerical abilities. Despite an abundance of researches on animals, cross-species comparison remains difficult because the methodology employed, the stimuli and the context of species examination vary considerably across studies. To date no study has investigated if different fish species have the same numerical systems. To fill this gap, the same procedure, stimuli, and numerical contrasts were used to compare quantity abilities of five teleost fish commonly used as model species in behavioral and cognitive studies: redtail splitfin, guppies, zebrafish, Siamese fighting fish, and angelfish. Fish initially were trained on an easy numerical ratio (5 vs. 10 and 6 vs. 12). Once they reached the learning criterion, they were subjected to non-reinforced probe trials in which the set size was constant but numerical ratios varied (8 vs. 12 and 9 vs. 12). They also were subjected to probe trials in which the ratio was constant, but the total set size was increased (25 vs. 50) or decreased (2 vs. 4). Similarities in numerical abilities in different species would support the hypothesis of cognitive mechanisms inherited from a common ancestor.

In the third and fourth studies it has been investigated whether numerical acuity could be affected by two factors that commonly occur in nature: collective behavior and movement of the items. In natural environment individuals interact with each other and these repeated interactions among grouping animals can influence adaptive response. Furthermore animals are naturally exposed to items in motion (prey, predators) and hence the movement represents a relevant cue in their life. However no studies have investigated whether fish can discriminate two-dimensional figures in motion and whether the accuracy is the same in the small and large number range.

The third study assessed whether the collective behavior may improve numerical acuity in guppies. In many ecological situations the capacity to process numerical magnitudes is important for an accurate decision-making but animals are often severely constrained in their ability to elaborate numerical information. However, it has been reported that, in some contexts, collective actions allow to bypass the cognitive limits of a species and to solve problems that go beyond the capacity of a single individual. Two experiments aimed to verify whether fish in dyads were more accurate than single individuals and could discriminate numerical ratios that singletons could not. In Experiment 1, the performance of dyads was confronted with that of singletons in a task requiring to join the larger shoal (4 vs. 6). In Experiment 2, fish were trained to discriminate between sets of figures (0.5 ratio) and hence were tested singly or in dyads in discriminations of increasing difficulty (8 vs. 12 and 9 vs. 12).

Finally, the fourth study investigated whether numerical acuity of fish is improved in the presence of items in motion. There is controversy in comparative psychology about whether the non-symbolic number estimation of small (≤4) and large numbers (≥ 4) involves a single mechanism (the ANS), or whether the enumeration of the numbers 1–4 is accomplished by a separate mechanism, the OTS. To date, support for the latter hypothesis has come only from the different ratio-dependency of performance seen in the two numerical ranges, a reading that has been criticized on several grounds. In humans, the two-system hypothesis is supported by evidence showing that manipulation of the physical properties of the stimuli (e.g., the motion of the items) has dissimilar effects on small- and large-number discrimination. This research studied the effect of items in motion on adult guppies. Initially, fish were trained to simultaneously discriminate two numerical contrasts having the same easy ratio (0.50): one in the small-number range and one in the large-number range. Half of the fish were presented with moving items; the other half were shown the same stimuli without motion. Fish were then subjected to non-reinforced probe trials in the presence of a more difficult ratio (0.75). A difference in fish ability to discriminate between small and large sets may provide further evidence of two separate numerical systems in fish.

The second part of the thesis is focused on the development of numerical abilities in newborn guppies. The study of cognitive abilities generally requires that subject are assessed individually, but social isolation can be stressful, especially for newborns and young individuals. The first study aimed to design a learning protocol for newborn guppies based on knowledge of their social needs. Firstly, the development of social behavior in the first two weeks of life was investigated by using a spontaneous choice task where newborn guppies could choose between social companions and an empty compartment. Then newborns were given the choice between a group of peers and their own mirror image to investigate whether mirrors could be used as a substitute for social companions during learning experiments. Based on the findings of these experiments, the protocol for discrimination learning in adult fish was adapted to study shape discrimination in newborn fish.

The ability to estimate shoal size was previously found to be inborn in guppies, although it improves with age and experience. However, it is not clear whether animals possess a single system of numerical representation, or rather are equipped with domain-specific numerical systems with distinct characteristics. To this purpose, the aim of the last study was to investigate whether newborn guppies could be trained to discriminate between groups of two-dimensional geometric figures differing in number (1 vs. 4, 2 vs. 4 and 2 vs. 3) and whether exhibit the same performance previously observed in the spontaneous shoal choice. To study the influence of continuous quantities, three different experiments were set up: in the first one number and continuous quantities were simultaneously available, in the second experiment only numerical information was available and in the last one, numerical information was made irrelevant (3 vs. 3) and only continuous quantities were available. Differences in developmental trajectories in comparison with shoal discrimination experiments would suggest the presence in fish of multiple quantification mechanisms which are domain-specific and serve to solve a limited set of problems.

3. THE SPECIES

Gambusia holbrooki

Regno	Animalia
Phylum	Chordata
Classe	Actinopterygii
Ordine	Cyprinodontiformes
Famiglia	Poeciliidae
Genere	Gambusia
Specie	*Gambusia holbrooki*

Fig. 12: *Male (below) and female (above)* of *Gambusia holbrooki.*

The eastern mosquitofish (*Gambusia holbrooki)* is a live-bearer fish native to North America and introduced in Europe at the beginning of XX century. It has been widely introduced as mosquito control agent and now it is described as the most widespread freshwater fish in the world. This species lives in shallow water with rich vegetation, but it is resistant to harsh conditions (low oxygen concentrations, high salt concentrations and high temperatures for short periods). Mosquitofish are omnivorous though the diet is based primarily on insect larvae.

It is a social species but males are less gregarious than females.

Sexual dimorphism is pronounced in body size but not in coloration: females are larger than males and adult female mosquitofish can be identified by a gravid spot they possess on the posterior of their abdomen (Fig. 12).

Fertilization is internal. Mosquitofish are viviparous: females give birth to newborns that are well developed and independent at birth. There is no parental care.

This species has been used as animal model in ecology (Howe et al., 1997; Toft et al., 2003; Hamer et al., 2002) and numerical cognition (Agrillo et al., 2008a, 2009, 2010, 2011)

Poecilia reticulata

Regno	Animalia
Phylum	Chordata
Classe	Actinopterygii
Ordine	Cyprinodontiformes
Famiglia	Poeciliidae
Genere	Poecilia
Specie	*Poecilia reticulata*

Fig. 13: *Male (above) and female (below) of Poecilia reticulata.*

The guppy (*Poecilia reticulata*) is a live-bearer fish native to Venezuela, Guyana, Barbados, Trinidad and Tobago. This species is widespread in different habitat but tends to be more abundant in smaller streams and pools than in large, deep, or fast flowing rivers. The guppies are omnivorous.

It is a social species though males are less gregarious than females.

Sexual dimorphism is pronounced: females are larger than males but males more are brightly coloured than females: males show a complex colour pattern polymorphism which consists of spots or patches varying in colour (orange, red, yellow, black; blue and green, Fig. 13).

Fertilization is internal and the species is viviparous: females gives birth to newborns that are well developed and independent at birth. There is no parental care.

This species is an important animal model to study sexual selection (Houde & Endler, 1990; Magurran et al., 1994; Pilastro et al., 2007), sociality (Laland & William, 1997; Magellan & Magurran, 2009; Croft et al., 2004) and it has been recently used to study numerical cognition (Agrillo et al., 2012, Bisazza et al., 2010)

Xenotoca eiseni

Regno	Animalia
Phylum	Chordata
Classe	Actinopterygii
Ordine	Cyprinodontiformes
Famiglia	Goodeidae
Genere	Xenotoca
Specie	*Xenotoca eiseni*

Fig. 14: *Male (above) and female (below) of Xenotoca eiseni.*

The redtail splitfin (*Xenotoca eiseni*) is a live-bearer fish native to Mesa Central plateau of Mexico. This species lives in shallow, quiet, transparent water with rich vegetation and pebbles (though fish rapidly adapt even to different environments) and is omnivorous.

It is a social species though males are less gregarious than females.

Females are generally slightly larger than males, but there is a strong dimorphism in the coloration, with males more brightly coloured than females (in males, but not females, the front part of the caudal peduncle is blue, and the area adjacent to the caudal fin is bright red, Fig. 14).

Fertilization is internal. Redtail splitfin is viviparous and females gives birth to newborns that are well developed and independent at birth. There is no parental care.

This species has been used to study spatial orientation (Sovrano et al., 2003, 2007), visual perception (Sovrano & Bisazza, 2008; Truppa et al., 2010) and numerical cognition (Stancher et al., 2013).

Betta splendens

Regno:	Animalia
Phylum:	Chordata
Classe:	Actinopterygii
Ordine:	Perciformes
Famiglia:	Osphronemidae
Genere:	Betta
Specie:	*Betta splendens*

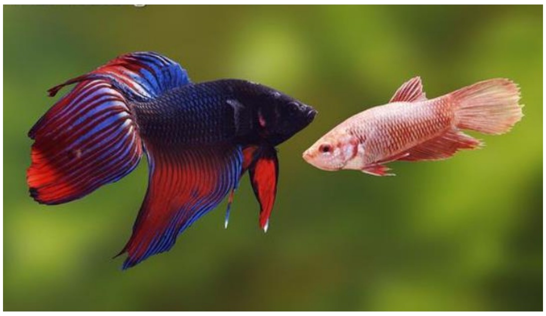


Fig. 15: *Male (left) and female (right) of Betta splendens.*

The Siamese fighting fish (*Betta splendens*) is native to Southeast Asia. It lives in rice paddies and marshy areas with a low oxygen content where can live as this species has an organ, called the labyrinth, that enables to breathe air through the surface of the bodies. The Siamese fighting fish are primarily carnivorous (crustaceans, larvae of mosquitoes and other insects).

It is a non-social species and both males and females are aggressive to each other.

Fish are sexually dimorphic: adult males are distinguished by larger bodies and longer fin length from the smaller, shorter finned females (Fig. 15).

Fertilisation is external and parental care is engaged by the male. Males build bubble nests at the water surface among floating plants and during and after spawning, they retrieve sinking eggs and deposit them in the bubble nest. After spawning the female is chased away from the male's territory and the eggs remain in the male's care. He keeps them in the bubble nest, making sure none falls to the bottom, repairs the nest and guard it from intruders.

The Siamese fighting fish has been primarily used in behavioral studies (Evans , 1985; Doutrelant et al., 2001, McGregor et al., 2001) but it is poorly investigated in comparative cognition.

Pterophyllum scalare


Fig. 16: *Sub-adults of Pterophyllum scalare*

Regno	Animalia
Phylum	Chordata
Classe	Actinopterygii
Ordine	Perciformes
Famiglia	Cichlidae
Genere	Pterophyllum
Specie	*Pterophyllum scalare*

The angelfish (*Pterophyllum scalare*) is a cichlid fish native to South America. This species lives in shore areas along the banks of lakes and slow-moving rivers with rich vegetation and roots, and fish are omnivorous.

The angelfish lacks sexual dimorphism and the difference in the genital papillae is the only reliable way to distinguish between males and females (Fig. 16).

It is a monogamous social species: angelfish tend to mate with a single partner and pairs form long-term relationships.

Fertilization is external: the pair selects a spawning site (such as a broad-leaf plant) and thoroughly clean it about two or three days before actual spawning takes place, then the female lays eggs and the male fertilizes them. Both partners engage in prolonged parental cares of the eggs and of the fry for a few days after hatching.

This species has been used as animal model to study sociality (Gòmez-Laplaza & Gerlai, 2002, 2005) and numerical cognition (Gòmez-Laplaza & Gerlai, 2011a, b; 2012, 2013).

Danio rerio

Regno	Animalia
Phylum	Chordata
Classe	Actinopterygii
Ordine	Cypriniformes
Famiglia	Cyprinidae
Genere	Danio
Specie	*Danio rerio*

Fig. 17: *Male (above) and female (below) of Danio rerio*

The zebrafish (*Danio rerio*), is a small, shoaling cyprinid, native to Northeast India, Bangladesh and Nepal, where it lives in shallow, slow-flowing waters, and rice paddies but also in rivers and hill streams. The zebrafish is an omnivorous and social species.

They are characterised by a distinctive colour pattern based on alternating dark and light horizontal stripes, which may be broken up into blotches or bars and they do not display striking sexual dimorphism (Fig. 17).

Fertilisation is external, the eggs are released directly over a substratum with no preparation by either sex and there is no parental care. Development is rapid and larvae display food seeking and active avoidance behaviors within 2-3 days after hatching.

The zebrafish is an important model organism in developmental genetics, biomedicine, neurophysiology, (Lieschke & Currie, 2007; Rubinstein, 2003; Amsterdam & Hopkins, 2006, Best & Alderton, 2008) and in behavioral studies (Miklósi & Andrew, 2006).

4. NUMERICAL ABILITIES IN ADULT FISH USING A TRAINING PROCEDURE

The study of animal cognition and its neurobiological bases often requires the adoption of associative learning procedures. Although fish are increasingly used as a model system in behavioral and cognitive studies, the availability of adequate learning protocols can be a limiting factor in this field of research. This Section describes a novel training procedure adopted in further studies to explore number discrimination in several teleost fish.

4.1. A new training procedure for studying discrimination learning in fish*

**This research is a modified version of "Agrillo, C., Miletto Petrazzini, M. E., Piffer, L., Dadda, M., & Bisazza, A. (2012). Behavioural Brain Research, 230, 343-348".*

During the past decade, an ever-increasing number of studies have used fish as a model system in behavioral neuroscience. Zebrafish, guppies, goldfish, and stickleback, among others, have been successfully employed to study learning, memory, and visual perception, to screen new drugs, to identify the function of brain genes, and to model human psychopathology and neurodegenerative diseases (Portavella et al., 2002; Burns et al., 2008; Sanogo et al., 2011; Geling et al., 2002; Hoffmann et al., 2007; Blank et al., 2009).

Many of these studies require visual discrimination. Although procedures of classical and operant conditioning used in avian and mammalian species can be adapted for fish (Siebeck, 2009; Bitterman, 2009; Yager, 1967) to learn to discriminate, they have several limitations. For example, with fish, water cannot be used as a reward, and food deprivation is not as effective as with warm-blooded vertebrates. Consequently, fish can only be administered a few trials per day and experiments may last several weeks.

Recently, some studies have used social reinstatement as a reward. Sovrano et al. (2002) studied the ability of fish to use the geometry of the environment for spatial reorientation. In this study, one fish was placed in an unfamiliar place and could only re-join its shoal mates by choosing the correct exit door. More recently, the same method was employed to study numerical abilities in mosquitofish (Agrillo et al., 2009; Agrillo et al., 2010). Al-Imari and Gerlai (2008) used social reinforcement successfully to train zebrafish to choose the arm associated with a red cue card in a four-arm radial maze. However, fish tend to become used to these procedures and the reinstatement tendency decreases after repeated testing. Hence, even with social reinforcement, the number of consecutive trials that can be performed is usually limited and the process of learning can last several weeks (Agrillo et al., 2010; Sovrano et al., 2008). In addition, the conditioning procedure requires that each subject is moved back and forth between the housing tank and the test apparatus several times a day, a procedure that is potentially very stressful for fish.

This study presents a novel procedure for training small fish to discriminate between two visual stimuli. In brief, for the entire experiment, each fish resides in a small tank that serves as testing apparatus. The stimuli to discriminate are repeatedly placed at the two ends of the tank while food is delivered in the proximity of the rewarded stimulus. The capacity to discriminate is measured as the time spent near the reinforced stimuli during probe trials without a reward.

To compare the new procedure with existing methods, we replicated recent published experiments that used operant conditioning to assess the limits of numerical discrimination in mosquitofish (Agrillo et al., 2009, 2010). As some authors (Feigenson et al., 2004; Agrillo et al., 2007, 2008a; Bonanni et al., 2011) have suggested that discrimination of small numbers (ranging from one to four) may be based on other mechanisms than discrimination of large numbers (≥ 4), we performed separate experiments for the two numerical ranges. In the first two experiments, we studied the influence of ratio and the influence of total size of the set on large number discrimination, respectively. In the third experiment, we studied the limit of discrimination in the small number range. In the fourth experiment, we tested whether extended training can improve the ability of fish to discriminate numerosities.

4.1.1. Experiment 1: Influence of numerical ratio in large number discrimination

This experiment investigated whether the discrimination of two large numbers worsens as the numerical ratio between the numerosities decreases, as previously reported using operant conditioning (Agrillo et al., 2010). To this end, fish were observed in their capacity to discriminate: 7 vs. 14, 8 vs. 12, and 9 vs. 12, which yielded ratios of 1:2, 2:3, and 3:4, respectively.

Subjects

Subjects were 11 adult female mosquitofish (ranging from 4 to 6 cm in length) of the species *Gambusia holbrooki*. Fish were initially collected from Valle Averto, a system of brackish water ponds and ditches in the Venetian lagoon basin. They were transported to the Laboratory of Comparative Psychology at the University of Padua and maintained for one month in 150 one-stock aquaria containing mixed-sex groups (15 individuals with approximately a 1:1 sex ratio). Aquaria were provided with natural gravel, an air filter, and live plants. Both stock aquaria and experimental tanks were maintained at a constant temperature of 25 ± 1◦ C and a 14:10 h light:dark (L:D) photoperiod with an 18-W fluorescent light. Before the experiment, fish were fed twice daily to satiation with commercial food flakes and live brine shrimp (*Artemia salina*).

Apparatus

The experimental apparatus was composed of a 50 x 19 x 32 cm tank. It was filled with gravel and 25 cm of water. The long walls were covered with green plastic material, and the short walls were covered with white plastic material. To reduce the potential effects of social isolation (see Section 6.1), two mirrors (29 x 5 cm) were placed in the middle of the tank, 3 cm away from the long walls. An artificial leaf (9 x 8 cm) was placed between the mirrors to provide some shelter for the subject. In correspondence with the sides in which stimuli were presented, two ‘choice areas’ were defined by white rectangles (14 x 12 cm) covered by a green net (Fig. 18).

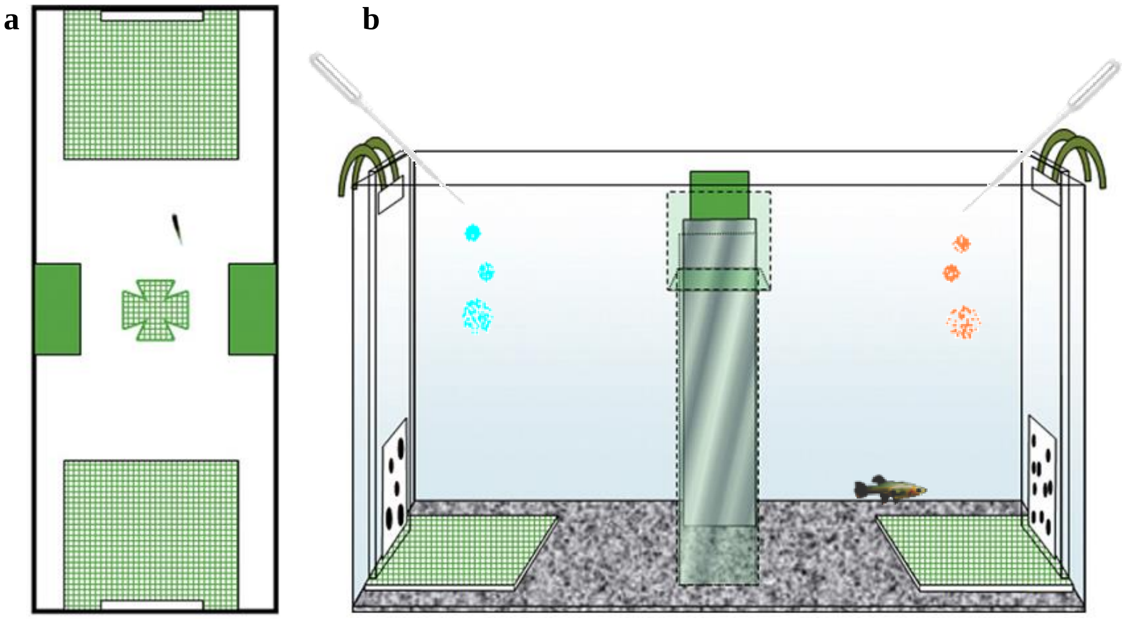


Fig. 18: *Experimental apparatus. Subjects were housed in the experimental tank (a: aerial view, b: lateral view) for the entire experiment. Stimuli (two groups of dots differing in numerosity) were presented at the bottom of the tank.*

Stimuli

Stimuli were inserted in a 6 x 6 cm square and were presented at the bottom of a 6 x 29 transparent plexiglass panel. They were groups of black geometric figures differing in size on a white background. Different numerical contrasts were presented: 5 vs. 10 and 6 vs. 12 (1:2 ratio) in the training phase; 7 vs. 14, 8 vs. 12, and 9 vs. 12 (1:2, 2:3, and 3:4 ratios, respectively) in the test phase. Stimuli selected for the experiment were extracted from a pool of 24 different pairs for each numerical contrast. Both the size and position of the figures were changed across sets to avoid the fish having to discriminate the overall configuration of the stimuli instead of using numerical information. It is known that numerosity co-varies with other physical attributes, such as cumulative surface area, overall space encompassed by the stimuli, and density of the elements; as well, it is known that human and non-human animals can use these non-numerical cues to estimate which group is larger/smaller (Gebuis & Reynvoet, 2012; Kilian et al., 2003; Pisa & Agrillo, 2009; Feigenson et al., 2002a). Cumulative surface area (summed area of dots) was controlled to reduce the possibility of fish using non-numerical cues. In particular, for one third of the stimuli, the two numerosities were 100 % equated for cumulative surface area. However, a by-product of equating the cumulative surface area was that smaller than average figures would be more frequent in the larger groups and fish might use this cue instead of number. To reduce this possibility, cumulative surface area was controlled to 85 % in another third of the stimuli during the training phase, and, in the remaining one-third of the stimuli, it was controlled to 70 %. As a consequence, in the 70 % condition the biggest figure within each pair was shown in the larger set; in the 85 % condition the biggest figure was shown in the larger set in half of the trials and in the smaller set in the other half of trials. In probe trials, cumulative surface area was always matched to 100 %. In this way, should the fish discriminate between the matched quantities in probe trials, neither cumulative surface area (matched to 100 %) nor the individual figure size (an unreliable cue in the training phase) could have played a key-role. In addition, given that density (inter-individual distance) is negatively correlated to the overall space occupied by the arrays (space encompassed by the most lateral figures), half of the set was controlled for overall space, whereas the second half was controlled for density (Fig. 19). Area, space and density were controlled using TpsDig software. Cumulative surface area, density, and overall space represent the non-numerical variables most frequently controlled in numerical cognition studies (Durgin, 1995; Pisa & Agrillo, 2009; Vos et al., 1988). They also represent the only cues that proved to be sometimes used by fish with static stimuli (Agrillo et al., 2009, 2010).

Eleven identical experimental tanks were used. They were placed close to each other on the same table and lit by two fluorescent lamps (36 W). A video camera was suspended about 1 m above the experimental tanks and used to record the position of the subjects during the tests.

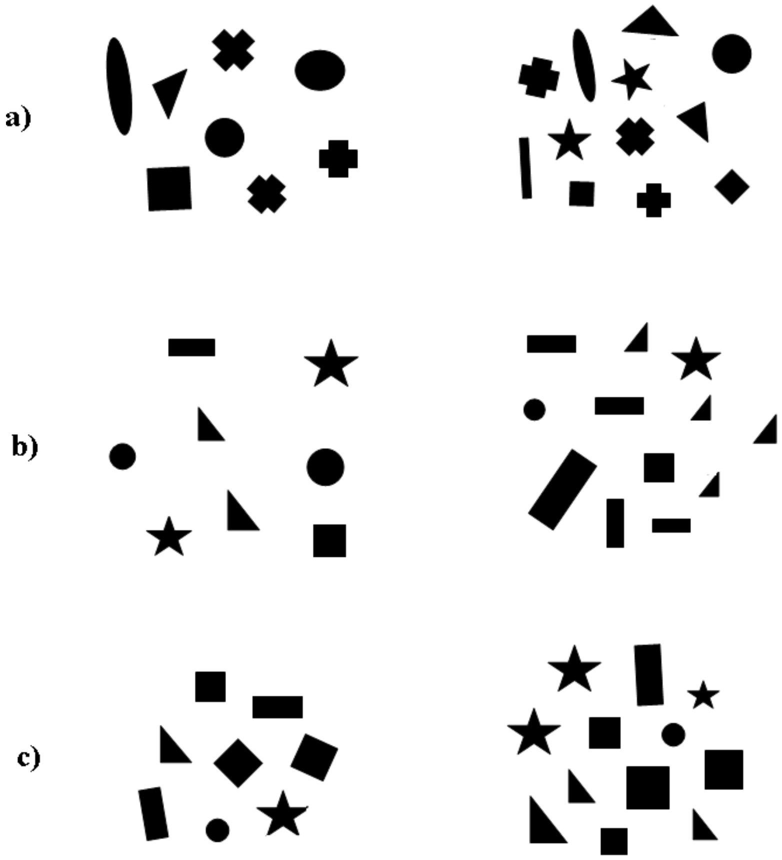


Fig. 19: *Schematic representation of the stimuli used. Each panel contained black geometric figures differing in numerosity inserted in a white background. In the figure we depicted a 8 vs. 12 contrast with cumulative surface area controlled to 100% (a), to 85% (b) and to 70%(c). In (a) and (b) stimuli are controlled for overall space, whereas in (c) they are controlled for density.*

Procedure

The experiment was divided into two different phases: training and test. During the training phase, we presented an easy numerical ratio (1:2) with the purpose of training the fish to the new task and selecting those fish successfully accomplished the task. In the test phase, we then assessed fish accuracy as the numerical ratio changed.

Training

In the two days preceding the start of the training, 11 fish were singly inserted into the experimental tanks in order to familiarize them with the tank. During this period, fish were fed twice a day. *Artemia* nauplii were inserted in the morning and in the afternoon near the two short walls.

On days 1–3, fish received four trials per day (three consecutive days, for a total of 12 trials). Each trial consisted of inserting the two stimuli hanged on the short walls. Two numerical contrasts were presented in a pseudo-random sequence: 5 vs. 10 and 6 vs. 12. Six fish were reinforced to the larger quantity and five fish to the smaller quantity. Soon after the stimuli were inserted in the tank, the experimenter used a Pasteur pipette to release the food reward (*Artemia* nauplii) in correspondence with the reinforced numerosity; an identical syringe was used to simultaneously insert pure water close to the non-reinforced numerosity. Subjects were left free to feed for 7 min. After this time, stimuli were removed from the tank. The inter-trial interval lasted 3 h. The left–right positions of the stimuli were counterbalanced over trials.

On days 4 and 5, two probe trials were alternated each day with two reinforced trials (four probe trials in total). In probe trials (two trials with 5 vs. 10 and two trials with 6 vs. 12, presented in a pseudo-random sequence), stimuli were inserted in the tank for 4 min; no reinforcement was provided (extinction procedure). The proportion of time spent by mosquitofish in the ‘choice areas’ was recorded as a measure of their capacity to discriminate the two numerosities (accuracy). In particular subjects were considered as selecting a stimulus when their heads were inside the choice area associated to that stimulus. Proportion of time was given by the following formula: (Time spent near the reinforced stimulus)/[(Time spent near the reinforced stimulus) + (Time spent near the non-reinforced stimulus)]. Reinforced trials were identical to those described for days 1–3.

Only fish who met the learning criterion (defined as 60% of the time spent near the reinforced numerosity in probe trials) were selected for the test phase.

To avoid the possibility of fish using the local/spatial cues of their tank, each subject was moved from one tank to another at the end of each day.

Interval

On days 6 and 7, fish received two reinforced trials each day, one in the morning and one in the afternoon. The same numerical contrasts of the training phase were presented in a pseudo-random sequence.

Test

Three probe trials were presented each day for four consecutive days (days 8–11). Fish were presented with three different numerical ratios, i.e., 1:2 (7 vs. 14), 2:3 (8 vs. 12), and 3:4 (9 vs. 12), and four presentations for each ratio in a pseudo-random sequence. The inter-trial interval lasted 3 h. Two reinforced trials presenting the numerical contrasts of the training (5 vs. 10 and 6 vs. 12) were alternated in the probe trials. The time spent in the ‘choice areas’ during probe trials was used as a dependent variable in this and in the following experiments.

Control test

As a by-product of controlling for the cumulative surface area, smaller than average elements were more frequent in the more numerous sets, and fish could have used this cue instead of numbers to learn discrimination. On day 12, fish were therefore subjected to a control test (7 vs. 14) involving pairs of stimuli composed of figures of identical size. Four probe trials were presented, alternating with two reinforced trials (5 vs. 10 and 6 vs. 12) in the following order: probe/probe/reinforced/probe/reinforced/probe.

Results

Training

In probe trials with a 1:2 numerical ratio, fish spent significantly more time near the trained numerosity (mean ± Standard deviation, SD: 0.609 ± 0.122; one sample *t*-test, *t*(10) = 2.967, *p* = 0.014). There was no difference in accuracy (proportion of time spent) between fish trained with the larger numerosities (0.586 ± 0.149) and those trained with the smaller numerosities as positive (0.637 ± 0.087; independent *t*-test, *t*(9) = 0.669, *p* = 0.520). Eight fish out of 11 met the learning criterion and hence started the test phase.

Test

Fish significantly discriminated in the 1:2 ratio (7 vs. 14, *t*(7) = 3.513, *p* = 0.010) and 2:3 ratio (8 vs. 12, *t*(7) = 2.815, *p* = 0.026), whereas their accuracy did not differ from chance level in the 3:4 ratio (9 vs. 12, *t*(7) = 1.056, *p* = 0.326, Fig. 20). A 2 x 3 ANOVA examining the effects of the Reinforced Numerosity (small/large) and Ratio (1:2, 2:3 and 3:4) revealed a main effect of Ratio (*F*(2,12) = 5.627, *p* = 0.019) with accuracy significantly decreasing with Ratio (linear trend: *F*(1,6) = 9.595, *p* = 0.021). Neither the main effect of Reinforced Numerosity nor the interaction between Ratio and Reinforced Numerosity were significant (*F*(1,6) = 0.082, *p* = 0.784, and *F*(2,12) = 0.204, *p* = 0.819, respectively).

Control test

In the presence of stimulus pairs composed of figures of identical size, fish significantly selected the trained numerosity (0.595 ± 0.061, *t*(7) = 4.367, *p* = 0.003).

4.1.2. Experiment 2: Influence of total set size in large number discrimination

This experiment aimed at studying whether the capacity to discriminate large numbers worsens as the total set size increases, as previously reported using operant conditioning (Agrillo et al., 2010). To this end, fish were observed in terms of their capacity to discriminate 8 vs. 16, 15 vs. 30, and 100 vs. 200.

Subjects and procedure

We tested the same subjects completed in Experiment 1. Fish were tested two days after the end of Experiment 1.

The general procedure was similar to the previous experiment. On days 1–3, four daily trials (three consecutive days, for a total of 12 trials) were performed. Three numerical contrasts with the same numerical ratio (1:2) but different total set size were presented: 8 vs. 16, 15 vs. 30, and 100 vs. 200 (four trials per numerical contrast).

On days 4–8, three probe trials were alternated each day with two reinforced trials (12 probe trials overall, four per each numerical contrast). The same numerical contrasts as on days 1–3 were presented.

Results

Fish significantly discriminated all numerical contrasts: 8 vs. 16 ($t(7) = 2.600$, $p = 0.035$), 15 vs. 30 ($t(7) = 3.935$, $p = 0.006$), 100 vs. 200 ($t(7) = 2.385$, $p = 0.049$, Fig. 20). A 2 x 3 ANOVA examining the effects of the Reinforced Numerosity (small/large) and Total Set Size (24, 45, and 300) revealed no main effects (Reinforced Numerosity: $F(1,6) = 0.255$, $p = 0.631$; Total Set Size: $F(2,12) = 0.133$, $p = 0.877$), or interaction ($F(2,12) = 1.716$, $p = 0.221$)

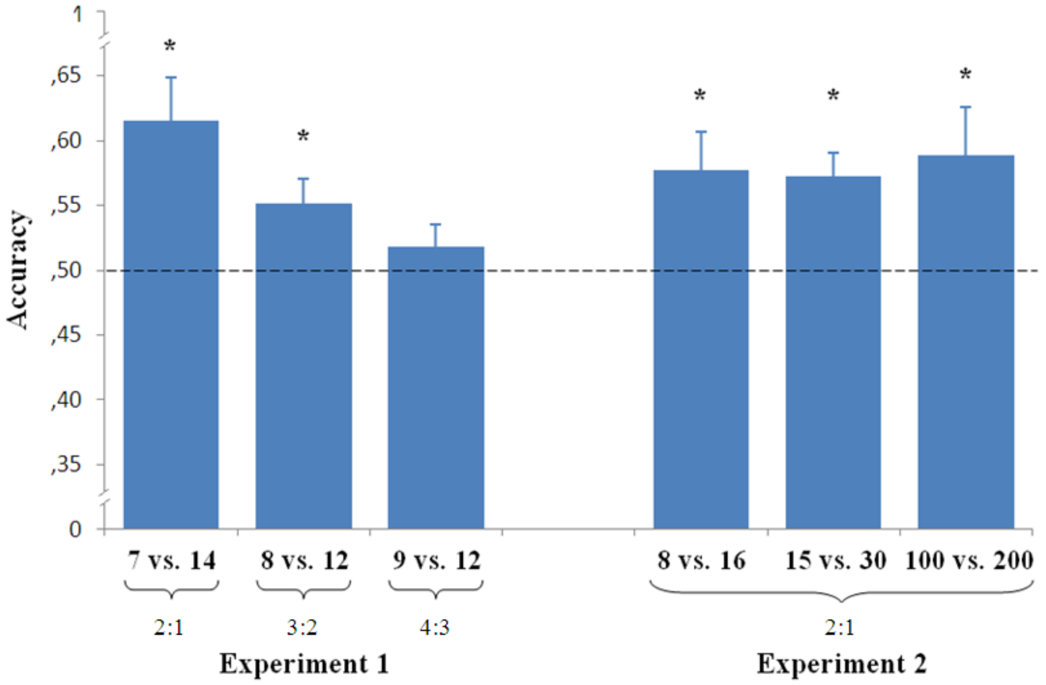


Fig. 20: *Results of Experiments 1 and 2. The method used in the present study successfully replicated the results of previous works using operant conditioning. The ability to discriminate between large numerosities shows ratio dependence (Experiment 1). Conversely, fish accuracy is insensitive to the total set size (Experiment 2). Asterisks denote a significant departure from chance level (P<0.05). Bars represent the standard error.*

4.1.3. Experiment 3: Limit of small number discrimination

This experiment aimed at studying the limit of precise number discrimination. To this end, fish were observed in their capacity to discriminate small groups differing by one unit (2 vs. 3, 3 vs. 4, and 4 vs. 5), as previously studied with operant conditioning (Agrillo et al., 2009)

Subjects

Subjects were 11 adult female mosquitofish (*G. holbrooki*). Before the experiment, fish were maintained in the same conditions described for Experiment 1.

Stimuli and procedure

The procedure was similar to Experiment 1. During the training phase, fish were presented (in both reinforced and probe trials) with the following numerical contrasts: 1 vs. 2 and 2 vs. 4. In the test phase, fish were presented with three novel numerical contrasts: 2 vs. 3, 3 vs. 4, and 4 vs. 5, four presentations for each numerical contrast. Two reinforced trials presenting 1 vs. 2 and 2 vs. 4 were alternated with the probe trials.

Results

Training

Fish significantly discriminated a 1:2 numerical ratio (mean ± SD: 0.591 ± 0.096; $t(10) = 3.146$, $p = 0.010$). There was no difference in the accuracy between fish trained with the larger numerosities (0.593 ± 0.114) and those trained with the smaller numerosities as positive (0.588 ± 0.081; $t(9) = -0.071$, $p = 0.945$). Seven fish out of 11 met the learning criterion and hence started the test phase.

Test

Fish significantly discriminated 2 vs. 3 (*t*(6) = 5.464, *p* = 0.002), but not 3 vs. 4 (*t*(6) = 0.470, *p* = 0.655), or 4 vs. 5 (*t*(6) = 0.154, *p* = 0.883, Fig. 21). There was a main effect of Ratio (*F*(2,10) = 5.378, *p* = 0.026, linear trend: *F*(1,5) = 12.073, *p* = 0.018) but no effect of Reinforced Numerosity (*F*(1,5) = 5.324, *p* = 0.069) was found. Interaction was not significant (*F*(2,10) = 0.364, *p* = 0.704).

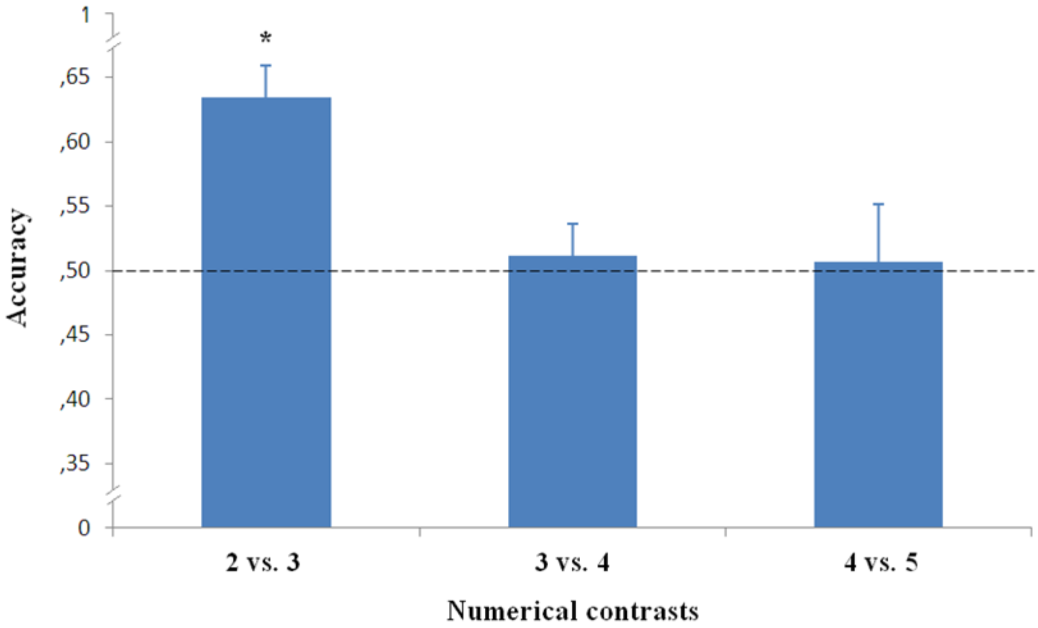


Fig. 21: *Results of Experiment 3. When trained to discriminate between groups differing by one unit, mosquitofish only prove able to successfully identify a 2 vs. 3 discrimination. Asterisk denotes a significant departure from chance level (P<0.05). Bars represent the standard error.*

4.1.4. Experiment 4: Influence of extended training on numerical discrimination

This experiment aimed at studying whether extensive training could improve the limits exhibited by mosquitofish in Experiment 3. To this end, we studied whether fish could improve discrimination of the numerical contrasts previously presented in the test phase of Experiment 3 (2 vs. 3, 3 vs. 4, and 4 vs. 5).

Subjects and procedure

We tested the same subjects who completed Experiment 3. Fish were tested two days after the end of Experiment 3. In week 1, subjects received 12 trials (four daily trials in days 1–3) with the same numerical contrasts used in the test phase of Experiment 3. As previously, in days 4–7, they were subjected to 12 probe trials (three daily probe trials alternated with two reinforced trials), four probe trials for each numerical contrast. An identical procedure was repeated during week 2.

Results

Fish re-tested after training on 2 vs. 3, 3 vs. 4, and 4 vs. 5 showed no variation in performance compared with Experiment 3. After 12 reinforced trials, they significantly discriminated 2 vs. 3 (0.590 ± 0.066, $t(6) = 3.603$, $p = 0.011$), but their performance did not differ from chance level in 3 vs. 4 (0.521 ± 0.067, $t(6) = 0.823$, $p = 0.442$), or 4 vs. 5 (0.504 ± 103, $t(6) = 0.115$, $p = 0.913$). No improvement was observed after 32 (12 trials + 8 alternated with probe trials in week 1 + 12 trials of week 2) trials (2 vs. 3: 0.589 ± 0.093, $t(7) = 2.548$, $p = 0.044$, 3 vs. 4: 0.514 ± 0.070, $t(7) = 0.560$, $p = 0.596$; 4 vs. 5: 0.511 ± 0.122, $t(7) = 0.248$, $p = 0.812$).

A repeated measures ANOVA with Sessions (Experiment 3/after 12/after 32 trials) and Numerical Ratio as within factors showed a main effect of Numerical Ratio ($F(2,12) = 8.602$, $p = 0.005$), whereas Sessions was not significant ($F(2,12) = 0.149$, $p = 0.863$). Interaction was not significant ($F(4,24) = 0.227$, $p = 0.921$).

Cumulatively, in Experiments 1 and 3, seven of the 22 fish did not meet the criterion to enter the subsequent test phase. One of these fish never reached the choice areas during probe trials, always remaining in the shelter, and three others had two or more invalid probe trials for the same reason (all 15 fish that met the criterion were valid in all four probe trials). This suggests that poor learning could be owed to a fear response in at least some fish. Indeed, the time spent on choice areas was significantly smaller in fish not meeting the criterion ($t(20) = 2.360$, $p = 0.029$).

4.1.5. Discussion

Existing training procedures for fish are often time-consuming and inefficient. In the present study, we developed an innovative procedure for training small fish to discriminate between two visual stimuli. Association time has been traditionally employed to evaluate both social preference and mate choice in fish (Buckingham et al., 2007; Fisher & Rosenthal, 2006). In the present study, we used association time near the positive stimulus as a measure of visual discrimination and tested the novel procedure by replicating recently published experiments on numerical abilities of mosquitofish (Agrillo et al., 2009, 2010).

The outcome of the new training method shows a complete overlap with the results we previously obtained using a conventional procedure of operant conditioning. As previously shown, mosquitofish proved able (Experiment 1) to discriminate between two large numbers with a ratio of 1:2 (7 vs. 14 items) or 2:3 (8 vs. 12), but not with a ratio of 3:4 (9 vs. 12). When the set size was varied (Experiment 2), the fish showed similar ability to discriminate 8 vs. 16, 15 vs. 30, or 100 vs. 200 elements, the same result found in the previously published experiment (Agrillo et al., 2010). When tested with small numbers (Experiment 3), fish showed the ability to discriminate 2 vs. 3 items, as reported by Agrillo et al. (2009) using operant conditioning, but not 3 vs. 4 or 4 vs.5 items.

One of the obvious advantages of this method is the rapidity of obtaining discrimination. In fact, in Experiments 1 and 3, we could demonstrate a significant choice of the positive stimulus only after the twelve reinforced trials of the training phase. As shown, fish in the training phase did not simply learn to discriminate the trained numerosities with a 1:2 ratio, but had apparently learned a general rule of choosing the larger (or smaller) numerosity, as they immediately extended their choice to new quantities with a 2:3 ratio. In other studies, subjects normally undergo many more trials to learn discrimination of similar difficulty (monkeys, approx. 5000 trials: Cantlon & Brannon, 2006; dolphins, approx. 3000 trials: Jaakkola et al., 2005; pigeons, approx. 1000 trials: Brannon et al., 2001), although much of this difference may be attributable to the different learning criterion adopted by the different studies.

Many more reinforced trials were also administered to fish in order to obtain numerical discriminations with the operant conditioning procedure adopted in the two studies we replicated here (Agrillo et al., 2009, 2010), a few hundred trials when one considers both training and pre-training phases of those experiments. As a reference, five days were necessary to train a group of fish to a simple numerical discrimination in the present study, whereas the same goal required a few weeks in previous studies (Agrillo et al., 2009, 2010).

Association with a reinforced stimulus has been recently used as a measure of learning in another study. Sison and Gerlai (2010) tested zebrafish using a plus maze, similar in design to the radial arm maze employed to study learning in rats and mice. Zebrafish received four daily trials for five consecutive days, during which they could find a food reward in one of four arms of the maze that was marked by a red cue card. When then tested in a single probe trial without food, fish showed a significant preference for the cued arm.

These results suggest that, in both mosquitofish and zebrafish, there may be an efficiency advantage in using a continuous measure of association instead of a classically discrete one, i.e., measuring time spent in association with a reinforced stimulus during one or a few probe trials, instead of computing the proportion of correct choices in many consecutive probe trials. Such a dependent variable however has been shown to be positively correlated with the proportion of correct choices in a pilot experiment using the above described procedure (Miletto Petrazzini et al., in preparation, unpublished data). With a traditional procedure, the learning criterion is normally established as 70–80% of correct choices in two consecutive series. Each series should contain no fewer than 10 probe trials (but more often larger figures are used) and probe trials should be mixed with a much larger number of reinforced trials. This easily raises the number of total trials necessary for the sole testing phase to several hundred, compared with a single (Sison & Gerlai, 2010) or a few probe trials (this study) with the new procedure. Since in our procedure the occurrence of discrimination is demonstrated through statistical inference (instead of a conventional individual threshold criterion), an adequate sample size is required. Therefore, this method appears less suitable for those studies that employ only one or a few subjects, such as the many studies that use large mammals (Kilian et al., 2003; Biro & Matsuzawa, 2001; Brannon & Terrace, 1998).

The procedure described here has some other advantages. Because it makes use of a very simple apparatus, a small glass tank, many subjects can be simultaneously tested. More importantly, by testing fish in their home tanks, we avoid the possible stress associated with moving the subject from its home tank to the testing apparatus and back in each experimental session. Further reduction of stress was obtained in our setup by two mirrors placed inside the tank. Many fish are in fact gregarious and may suffer if socially isolated. Several studies have shown that adult fish tend to interact with their own image as if they are in the presence of other conspecifics (Milinski, 1988; Thompson & Sturm, 1965, Meliska et al., 1980). Finally, unlike the traditional operant conditioning approach, our procedure did not require starving fish for protracted periods: our subjects were fed three or four times a day, thus receiving the same amount of food as they would receive during normal rearing.

One of the drawbacks of our method is that a consistent proportion of subjects (27–36%) did not meet the learning criterion in the training phase. For comparison, this normally occurred in fewer than 5% of the cases in previous mosquitofish experiments using other operant conditioning procedures (Agrillo et al., 2009, 2010). One obvious explanation for this finding is that we used too few trials in the training phase. In a recent experiment using this procedure with guppies, however, even after extensive training, we were unable to improve the performance of fish that did not meet the criterion after the first 12 trials (unpublished data). An analysis of time spent in choice areas by the seven mosquitofish that did not meet the criterion suggests that their poor performance was owed to a fear response to the procedure or to poor adaptation to the apparatus. Future experiments should perhaps contemplate a longer acclimatization period or an adaptation of fish to the apparatus in social groups (for example, see Al-Imari & Gerlai, 2008).

Some authors have argued that although training procedures are a powerful tool for investigating cognition and provide strong control over the stimulus conditions, extensive training can lead an animal to develop a learned ability that may not be present in the natural repertoire of the species (Hauser et al., 2000; Hauser & Spelke, 2004; Uller, 2008). Conversely, the study of spontaneous capacities, for instance the preference for different numbers of social companions or food items (Dadda et al, 2009; Hauser et al., 2000), may provide a better picture of what species do naturally and thus provide a deeper insight into adaptive functions. It must be pointed out that although our experiment involved an initial training phase, what we observed in the testing phase of Experiments 1 and 2 is indeed the spontaneous capacity to discriminate numerosities. For instance, in Experiment 1 we initially trained a fish to choose the larger (or smaller) quantity in a very easy discrimination task (1:2 ratio) and hence in the test phase we proposed new numerical ratios (2:3, 3:4) for the first time during probe trials (i.e., fish had no prior training on these tasks). Indeed, when we trained them to discriminate the same quantities for 30 trials (Experiment 4), we did not observe any improvement in their baseline capacity. Since this training is much longer than that necessary to obtain a 1:2 discrimination, it appears that spontaneous and trained numerical capacities are coincident in mosquitofish. This does not exclude the possibility that with a different procedure mosquitofish could be trained to demonstrate finer numerical discrimination. Indeed in another context, spontaneous choice of the larger social group, mosquitofish have proved able to discriminate three from four fish, an ability not shown here (Agrillo et al., 2007, 2008a).

As such, the paradigm described in this paper is feasible without special skills or tools, but the protocol is also well suited for automation. Stimulus delivery on a computer screen could be synchronized with automated tracking of the fish movements using one of the available programs (e.g., Willis et al., 2011; Verzijden & Rosenthal; 2011), which could also serve for the automated measurement and analysis of visual choice. This might provide a system for high-throughput conditioning of fish in a manner similar to approaches already used with rodents and offer a powerful tool in many fish studies involving learning.

Even though investigating quantity abilities was not a primary goal of this study, the observation in Experiment 3 that mosquitofish can be trained to discriminate between two groups of figures differing by one to three units (1 vs. 2 and 2 vs. 3, but not 3 vs. 4 or 4 vs. 5) agrees with the limit reported in other non-verbal organisms (infants: Feigenson et al., 2002a; horses: Uller & Lewis, 2009; domestic chicks: Rugani et al., 2008; bees: Gross et al., 2009). This result contrasts however with previous findings that mosquitofish can select the larger shoal in a 3 vs. 4 discrimination task (Agrillo et al., 2007, 2008a). One possible explanation is that numerical abilities vary with context and that perhaps accuracy in discriminating social groups is under stronger selective pressure since the ability to select larger shoals strongly affects susceptibility to predation (Hager & Helfman, 1991; Pritchard et al., 2001; Hoare et al., 2004). However, since we did not control for non-numerical variables (cumulative surface area, luminance, density and the overall space occupied by the groups) in shoal choice experiments, the better performance with this paradigm could also derive from the possibility for fish to use multiple cues for discriminating stimuli (Agrillo et al., 2011).

In conclusion, although several questions remain unanswered and some of the details of the method will have to be refined, our study paves the way for developing reliable procedures for quick acquisition of discrimination in fish that are an alternative to the long and often inefficient training procedures presently in use.

4.2. Inter-specific differences in numerical abilities among teleost fish*

**This research is a modified version of "Agrillo, C., Miletto Petrazzini M. E., Tagliapietra, C., & Bisazza, A. (2012). Frontiers in Psychology, 3:483".*

Though numerical abilities were once considered a unique human ability, numerous studies have now shown that other primates display the capacity to add, subtract and order numerical information (Beran, 2004; Brannon & Terrace, 1998; Matsuzawa, 2009). The evidence collected in cognitive, developmental and comparative research has led several authors to propose that adults prevented from verbal counting, infants and non-human primates possess similar numerical systems that are independent from language and culture (Beran, 2008a; Feigenson et al., 2004; Hauser & Spelke, 2004). For instance, the performance of rhesus monkeys adheres to that of adult humans in two comparative studies where both species were presented identical stimuli (Cantlon & Brannon, 2006, 2007b). In chimpanzees, error rates and reaction times are constant in the subitizing range (1–4) while they increase monotonically for larger numbers, suggesting the existence of a subitizing-like mechanism in apes (Tomonaga & Matsuzawa, 2002).

Following the discovery of the remarkable numerical skills of primates, researchers initially believed in the existence of a sharp discontinuity in cognitive abilities between primates and other animal species. However, during the last decade, the presence of basic quantity abilities has been reported in other mammals (bears: Vonk & Beran, 2012; elephants: Perdue et al., 2012; dogs: West and Young, 2002; dolphins: Kilian et al., 2003), in birds (parrots: Pepperberg, 2006; Al Aïn et al,, 2009; pigeons: Roberts, 2010), in fish (mosquitofish: Agrillo et al., 2009; angelfish: Gómez-Laplaza & Gerlai, 2011a, b; swordtails: Buckingham et al., 2007), and even in invertebrates (ants: Reznikova & Ryabko, 2011; bees: Gross et al., 2009; beetles: Carazo et al., 2009).

Such new evidence has prompted a debate as to whether or not all species share the same quantity systems. Some studies have reported interesting similarities between primates and non-primate species. For instance, different mammals (Perdue et al., 2012;

Ward & Smuts, 2007), birds (Al Aïn et al., 2009), amphibians (Krusche et al., 2010), and fish (Gómez -Laplaza & Gerlai, 2011b) are affected by the numerical ratio when required to compare numerosities, as commonly reported in primates (Beran, 2004; Cantlon & Brannon, 2007b). Rhesus monkeys can discriminate 1 vs. 2, 2 vs. 3, and 3 vs. 4, but not 4 vs. 5 (Hauser et al., 2000), the same limit exhibited by distantly related species, such as Eastern mosquitofish (Agrillo et al., 2008a), guppies (Agrillo et al., 2012) and robins (Hunt et al., 2008). Domestic chicks and robins can make spontaneous use of numerical information instead of using non-numerical perceptual cues that co-vary with number, such as cumulative surface area or density (Hunt et al., 2008; Rugani et al., 2009), which aligns with what has been reported in human (Cordes & Brannon, 2008; Nys & Content, 2012) and non-human primates (Cantlon & Brannon, 2007a). Similar performance in the discrimination of small and large numbers recently has been reported in a study comparing humans and guppies (Agrillo et al., 2012).

However, many other studies have evidenced that performance varies across species in many respects. For example -unlike primates, chicks and robins- cats and dolphins seem to use numerical information only as a ‘last-resort’ strategy, when no other continuous information is available (Pisa & Agrillo, 2009; Kilian et al., 2003). Horses, chicks, salamanders and angelfish seem to be able to discriminate between groups differing by one unit up to 2 vs. 3 items (Rugani et al., 2007; Uller & Lewis, 2009; Uller et al., 2003; Gómez-Laplaza & Gerlai, 2011a), while mosquitofish, guppies and robins discriminate up to 3 vs. 4 items (Agrillo et al., 2008a, 2012; Hunt et al., 2008). Trained pigeons can discriminate up to 6 vs. 7 items (Emmerton & Delius, 1993), well above the limit of number discrimination of 2 vs. 3 items observed in trained chicks (Rugani et al., 2007). Differences have been reported even between closely related species. For example, the ability to discriminate between large quantities appears to be affected by numerical ratio in African elephants (Perdue et al., 2012), but not in Asian elephants (Irie-Sugimoto et al., 2009).

Despite the wealth of comparative data, cross-species comparison has been difficult because the tasks investigated, the methodology employed, the sensory modality involved, and the context of species investigation vary considerably from one study to another. In some cases, the inconsistency is clearly to be ascribed to the different methods adopted. For example, when required to discriminate 1 vs. 2 and 2 vs. 3, the numerical ratio plays a key role in infants' performance using auditory stimuli (vanMarle & Wynn, 2009), but not visual stimuli (Feigenson et al., 2002a). Similarly, in goldbelly topminnows, the performance in a quantity discrimination task was affected by the type of procedure, with fish able to discriminate 2 versus 3 only in one of two different procedures (Agrillo & Dadda, 2007).

To date, cross-species comparison using the same methodology rarely has been attempted; such studies have primarily related to primates (Cantlon & Brannon, 2006, 2007b; Hanus & Call, 2007). To fill this gap, the present study compares the numerical abilities of five teleost fish (redtail splitfin, guppies, zebrafish, Siamese fighting fish and angelfish) using the same procedure, stimuli and numerical contrasts. Subjects were trained with a food reward to discriminate between two sets of geometrical figures differing in numerosity. Fish initially were trained on an easy numerical ratio (0.50). Once they reached the learning criterion, they were tested in non-reinforced probe trials for their ability to generalize to more difficult ratios (0.67 and 0.75), or to a larger or a smaller total set size. In addition, because the proportion of individuals reaching the initial learning criterion was very low in one species, we conducted a control experiment on shape discrimination to assess if this difference was specific to the numerical domain or was due to a more general cross-species difference in learning with this procedure.

4.2.1. Experiment 1: Influence of numerical ratio and total set size in different fish species

Subjects

The initial subjects of this experiment were 16 *Xenotoca eiseni* (redtail splitfin, total length: mean ± SD: 3.02 ± 0.25), 16 *Poecilia reticulata* (guppies, 2.01 ± 0.30), 16 *Betta splendens* (Siamese fighting fish, 3.36 ± 0.32), 16 *Pterophyllum scalare* (angelfish, 4.09 ± 0.38) and 16 *Danio rerio* (zebrafish, 2.84 ± 0.27). All subjects were adult females with the exception of the group of angelfish composed by unsexed juvenile individuals. Redtail splitfin came from the stocks maintained in our lab; guppies were also maintained in our lab and were fifth generation descendants of wild-caught fish from the Tacarigua River in Trinidad. Siamese fighting fish, angelfish and zebrafish were obtained from local commercial suppliers. As few zebrafish reached the criterion, we increased the sample size for this species by adding 10 more specimens of the same strain (hereafter called “commercial stock”) and by testing 22 more specimens from another strain coming from the outbreed stock maintained at the Biology Department of University of Padua (hereafter called “lab stock”).

Subjects were stocked at the Laboratory of Comparative Psychology (University of Padua) for at least 15 days before the beginning of the experiments and maintained in 150 l stock aquaria; each species was housed separately. Aquaria were provided with natural gravel, air filters, and live plants. Both stock aquaria and experimental tanks were maintained at a constant temperature of 25 ± 1°C and a 14:10 h light:dark (L:D) photoperiod; stock aquaria were lit by an 18-W fluorescent light, experimental tanks were lit by two 36 W fluorescent lamps. Before the experiment, fish were fed twice daily to satiation with commercial food flakes and live brine shrimp (*Artemia salina*).

Apparatus and stimuli

We used the apparatus described in Section 4.1.

Stimuli were groups of black geometric figures differing in size on a white background. We presented different numerical contrasts: 5 vs. 10 and 6 vs. 12 (0.50

ratio) in the training phase; 8 vs. 12 and 9 vs. 12 (0.67 and 0.75 ratios) in phase 1; 2 vs. 4 and 25 vs. 50 in phase 2. Stimuli selected for the experiment were extracted from a pool of 24 different pairs for each numerical contrast. Both the size and position of the figures were changed across sets to avoid the fish having to discriminate the overall configuration of the stimuli instead of using numerical information. Stimuli were controlled for continuous quantities using the procedure described in Section 4.1.

Sixteen identical experimental tanks were used. They were placed close to each other on the same table. A video camera was suspended about 1 m above the experimental tanks and used to record the position of the subjects during the tests.

Procedure

The experiment was divided into three different steps: pre-training, training, and test.

Pre-training was set up to permit fish to familiarize themselves with the experimental apparatus. Subsequently, all fish were singly trained to discriminate an easy numerical ratio (0.50) with the purpose of selecting those fish that successfully accomplished the numerical task.

Fish who reached the criterion were subsequently tested with novel numerical contrasts. This was divided in two phases: in phase 1, we observed the ability to discriminate between large numbers with two different numerical ratios: 8 vs. 12 (0.67) and 9 vs. 12 (0.75); in phase 2, we assessed whether fish showed the ability to generalize the numerical rule to novel numerosities having the same ratio (0.50), but very different total set size (2 vs. 4 and 25 vs. 50).

Pre-training

During the six days preceding the beginning of training, fish gradually were familiarized with the apparatus. On days 1-2, groups of 4 subjects of the same species were inserted in the experimental apparatus for a total of 4 hours; on days 3-4, two subjects of each species were inserted in the apparatus for 4 hours, while on day 5-6, fish stayed singly in the apparatus for the whole day. During this latter period, fish were fed twice a day. *Artemia* nauplii. were inserted in the morning and in the afternoon near the two short walls.

Siamese fighting fish are known to be poorly social, as a consequence they were the only exception to this procedure. For this species, pre-training was identical but subjects were individually inserted in the apparatus starting from day 1.

Training

We followed the procedure described in Section 4.1.

On days 7 to 9, fish received four trials per day (three consecutive days, for a total of 12 trials). Two numerical contrasts were presented in a pseudo-random sequence: 5 vs. 10 and 6 vs. 12. We counterbalanced the position of the stimuli (left-right) over trials. Half of the fish were reinforced to the larger numerosities, while the second half was reinforced to the smaller numerosities.

On days 10 and 11, two probe trials were alternated with two reinforced trials each day (four probe trials). In probe trials no reinforcement was provided (extinction procedure). Reinforced trials were identical to those described for days 7 to 9. Only fish that met the criterion (defined as 60% of the time spent near the reinforced numerosity in probe trials) were selected for the test. The previous study has shown that, in easy tasks, such a criterion permits to distinguish fish that learn discriminations from those fish that continue to choose randomly (see Section 4.1).

Subjects were moved from one tank to another one at the end of each day in order to avoid the possibility of using the local/spatial cues of the tank. The latter was previously occupied by conspecific subjects.

Test

After a short interval (days 12-13) in which subjects received two reinforced trials each day with the same numerical contrasts presented during training, fish started the test. The test was composed of two different phases. In phase 1, three probe trials were presented each day for four consecutive days (days 14 to 17). Fish were presented with two different numerical ratios, 0.67 (8 vs. 12) and 0.75 (9 vs. 12), six presentations for each ratio in a pseudo-random sequence. The inter-trial interval lasted three hours. Two reinforced trials presenting the numerical contrasts of the training (5 vs. 10 and 6 vs. 12) were alternated in the probe trials.

In phase 2, four probe trials were presented each day for two consecutive days (days 18 to 19). Fish were observed for their ability to generalize to small (2 vs. 4) and large (25 vs. 50) numbers; there were four presentations for each ratio in a pseudo-random sequence. The numerical ratio was equal to 0.50.

In both phase 1 and 2, we considered the proportion of time spent in the ‘choice areas’ (accuracy) during probe trials as the dependent variable. Proportions were arcsine (square root)-transformed (Sokal & Rohlf, 1995). Mean ± SD are provided. Statistical tests were carried out using SPSS 18.0.

Results

Training

In zebrafish, 5 out of 26 fish in the commercial stock and one out of 22 of the lab stock reached the criterion. The two strains of zebrafish did not differ in performance (independent *t*-test, $t(46) = 1.48$, $p = 0.148$) and were pooled together in subsequent analyses. A total of 42 fish reached the criterion and were admitted to the following phases (10 out of 16 redtail splitfin, 8/16 guppies, 10/16 Siamese fighting fish, 8/16 angelfish and 6 out of 48 zebrafish). We found a significant difference among the species in the number of subjects reaching the criterion (chi square test, $\chi(4) = 23.48$, $p < 0.001$). This finding results from the fact that the number of individuals reaching the criterion in zebrafish was significantly lower compared to the other four species (zebrafish: 6/48, 12.5%; remaining 4 species: 36/64, 56.3%; chi square test, $\chi(1) = 22.4$ $p < 0.001$).

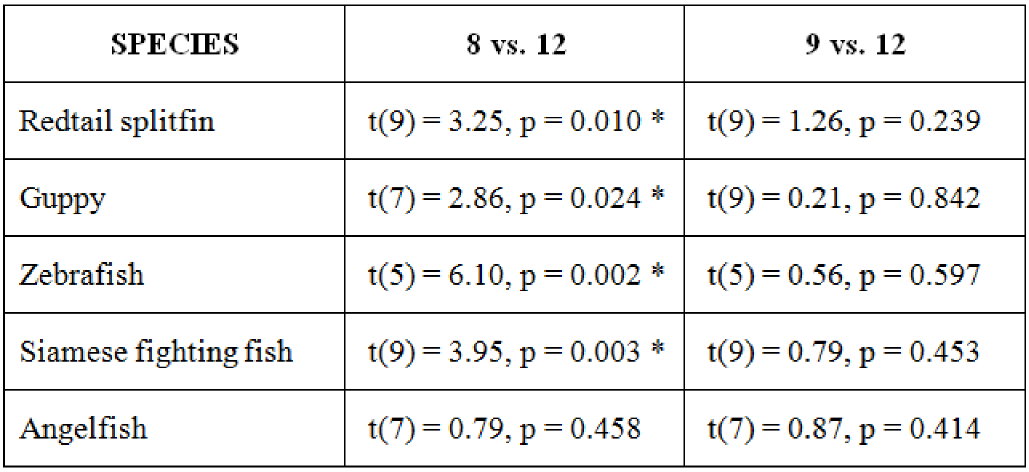
No difference between the species was found in the proportion of time spent in the choice areas (one way ANOVA, $F(4, 37) = 0.94$, $p = 0.452$). In particular, when analyzing the time spent in the choice areas of all individuals, no difference was found between zebrafish and the other species pooled together (independent *t*-test, $t(110) = 0.84$, $p = 0.400$).

Test

Phase 1: Influence of numerical ratio

We found no difference in the accuracy between fish trained with the larger or smaller numerosities as positive (independent *t*-tests, $p > 0.05$ for both ratios).

No species proved able to discriminate 9 vs. 12 items (Table 1). There was no difference in performance among the 5 species (one way ANOVA, $F(4, 37) = 0.45$, $p = 0.772$). All species, except angelfish, significantly discriminated 8 vs. 12 items (table 1). A significant difference among the five species was found for this ratio (one way ANOVA $F(4, 37) = 3.30$, $p = 0.021$). On the whole there was a significant difference between the two numerical ratios (repeated measure ANOVA, Ratio: $F(1, 37) = 9.42$ $p = 0.004$; species: $F(4, 37) = 1.59$, $p = 0.197$; interaction: $F(4, 37) = 0.70$, $p = 0.597$, Fig. 22).



SPECIES	8 vs. 12	9 vs. 12
Redtail splitfin	t(9) = 3.25, p = 0.010 *	t(9) = 1.26, p = 0.239
Guppy	t(7) = 2.86, p = 0.024 *	t(9) = 0.21, p = 0.842
Zebrafish	t(5) = 6.10, p = 0.002 *	t(5) = 0.56, p = 0.597
Siamese fighting fish	t(9) = 3.95, p = 0.003 *	t(9) = 0.79, p = 0.453
Angelfish	t(7) = 0.79, p = 0.458	t(7) = 0.87, p = 0.414

Table 1: *Performance of the five species in the numerical contrasts presented in test phase 1***.**

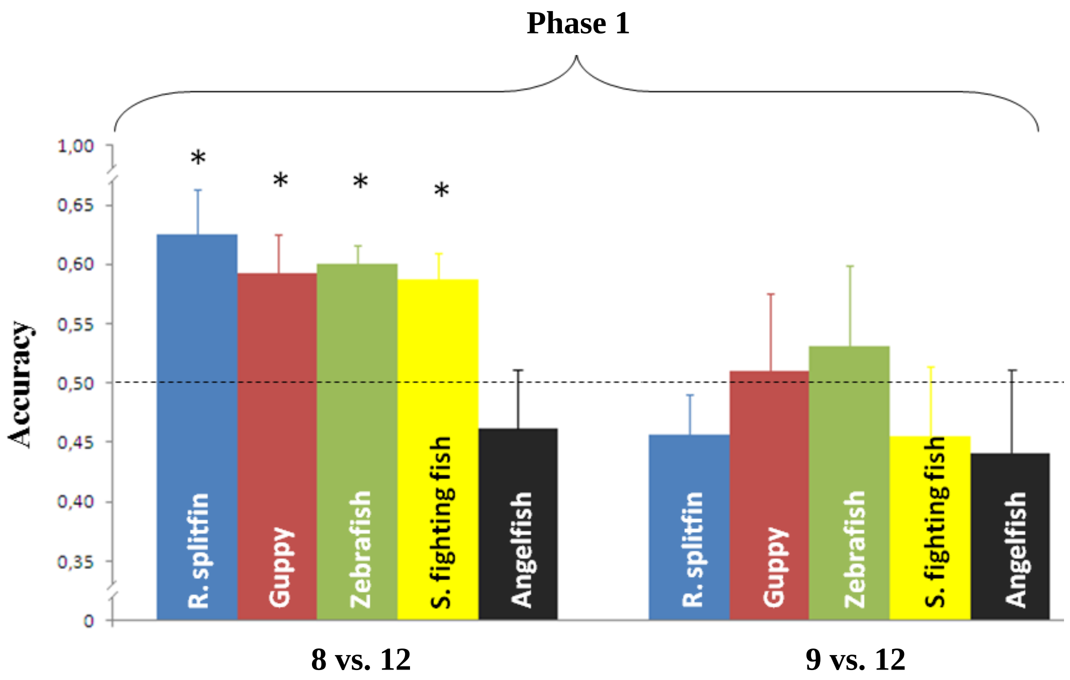


Fig. 22: *Numerical contrasts are plotted against the accuracy of the five species. Most of the species significantly discriminated 8 vs. 12 but all species failed to discriminate 9 vs. 12. Asterisks denote a significant departure from chance level (p < 0.05). Bars represent the standard error.*

<u>*Phase 2. Generalization to small and large numerosities*</u>

No species proved able to generalize the learned discrimination to a larger set size, 25 vs. 50 items (Table 2). There was no difference in performance among the five species (one way ANOVA, *F*(4, 37) = 0.06, *p* = 0.992). Three species (readtail splitfin, Siamese fighting fish and angelfish) significantly generalized the learned discrimination to a smaller set size, 2 vs. 4 items. One species (guppy) yielded a marginally significant result, and one species (zebrafish) was not significant (Table 2). However, the trend is similar in all 5 species, and the difference between them is not significant (one way ANOVA, *F*(4, 37) = 0.49, *p* = 0.741). A likelihood ratio analysis (see Glover & Dixon, 2004 for details) confirmed that the probability that the five species do not differ is three times larger (λ = 2.98) than the probability that a difference exists. Overall, the difference in the generalization between the larger and smaller set size was significant (repeated measure ANOVA, *F*(1, 37) = 9.84 *p* = 0.003) with no species variation (*F*(4, 37) = 0.23, *p* = 0.919; Interaction: *F*(4, 37) = 0.06, *p* = 0.911, Fig. 23).

We found no difference in the accuracy between fish trained with the larger or smaller numerosities as positive (2 vs. 4, independent *t*-test, *t*(40) = 1.34, *p* = 0.187; 25 vs. 50, independent *t*-test, *t*(40) = 0.22, *p* = 0.826).

SPECIES	**2 vs. 4**	**25 vs. 50**
Redtail splitfin	t(9) = 3.12, p = 0.012 *	t(9) = 0.18, p = 0.861
Guppy	t(7) = 2.02, p = 0.083	t(9) = 0.05, p = 0.961
Zebrafish	t(5) = 1.55, p = 0.181	t(5) = 0.42, p = 0.690
Siamese fighting fish	t(9) = 3.42, p = 0.008 *	t(9) = 0.40, p = 0.698
Angelfish	t(7) = 2.50, p = 0.041 *	t(7) = 0.76, p = 0.942

Table 2: *Performance of the five species in the numerical contrasts presented in test phase 2***.**

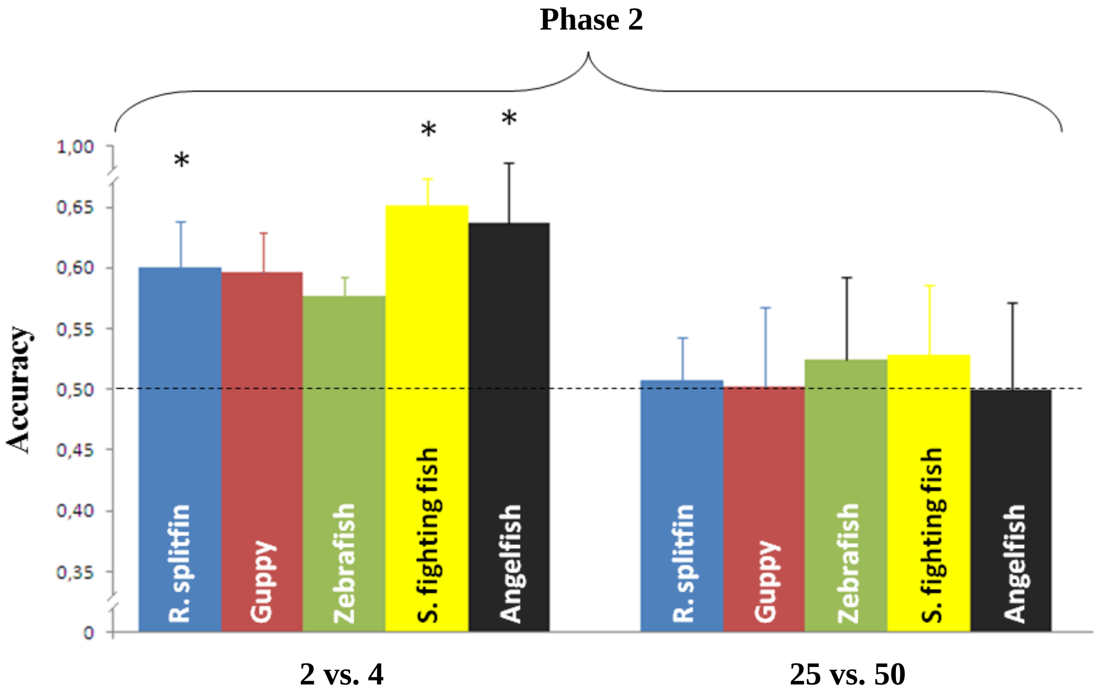


Fig. 23: *Numerical contrasts are plotted against the accuracy of the five species. Most of the species spontaneously generalized to smaller numerosities (2 vs. 4) but no species generalized to larger numerosities (25 vs. 50). Asterisks denote a significant departure from chance level (p < 0.05). Bars represent the standard error.*

4.2.2. Experiment 2: Control test: shape discrimination test

Subjects, apparatus, stimuli and procedure

Twenty fish (10 *D. rerio* and 10 *X. eiseni*) were tested. Both species were observed in a discrimination between two black geometric figures in a white background (filled triangle vs. empty circle). For each species, half of the subjects were reinforced to the triangle, and half to the circle. The same figures were presented during all trials (both training and probe trials). The apparatus was identical to that of Experiment 1. The procedure also was the same, with the exception that the experiment ended after the four probe trials of the training phase.

Results

We found no difference in the accuracy between fish trained with the triangle or circle as positive (independent *t*-tests, $p > 0.05$ for both species). Redtail splitfin significantly discriminated between the two figures (mean ± SD: 0.594 ± 0.06, one sample *t*-test, $t(9) = 4.65$, $p = 0.001$), while zebrafish did not (0.471 ± 0.08, one sample *t*-test, $t(9) = 1.08$, $p = 0.307$). A significant difference between the two species was found (independent *t*-test, $t(18) = 3.70$, $p = 0.002$). No difference in the accuracy was found between fish trained in numerical discrimination (training of phase 1) and those trained to discriminate geometric figures (independent *t*-test for unequal cases redtail splifin: $t(24) = 0.807$, $p = 0.428$; zebrafish: $t(56) = 0.005$, $p = 0.996$).

4.2.3. Discussion

The present study represents the first attempt to compare numerical abilities in teleost fish using the same methodology. Subjects of five teleost species first were trained to discriminate two sets of geometrical figures using an easy 0.50 numerical ratio (5 vs. 10 and 6 vs. 12) and then observed in non-reinforced probe trials in which the numerical ratios or total set size varied. Overall, similarities were far greater than differences. Fish trained with the larger or smaller numerosities as positive showed equal accuracy in all species. When we made the discrimination more difficult by increasing the numerical ratio, we observed a similar pattern of performance in all fish, with no species being able to discriminate the 0.75 ratio (9 vs. 12), but 4 out of 5 species being able to discriminate the 0.67 ratio (8 vs. 12). The pattern of generalization of the numerical rule to a different set size was also very similar in the different species. Fish generalized the learned discrimination to a smaller set size (2 vs. 4), showing no substantial inter-specific difference, while no species was able to generalize to a larger set size (25 vs. 50). These data, together with the results previously reported in mosquitofish tested in the same apparatus (see Section 4.1), point toward the existence of similar numerical discrimination among fish.

In all, we observed three main inter-specific differences. First, the proportion of subjects that reached the criterion in the training phase was similar among species, with the exception of zebrafish, which showed a 4-fold smaller proportion of fish reaching the criterion. This difference might be ascribed to two reasons: a) a specific deficit of zebrafish regarding numerical skills, or b) a more general inability of this species in discrimination learning. The results of Experiment 2 support the latter hypothesis. When trained to learn simple shape discrimination, a filled triangle from an empty circle, zebrafish performed much worse than the control species, redtail splitfin. In recent years, a few works have been published regarding the possibility of training visual discrimination in zebrafish. In most cases, the required discrimination was even more simple than this, i.e. to distinguish a red wall from other non-colored ones (Sison & Gerlai, 2010), or implied a much larger number of trials (Braubach et al., 2009). To our knowledge, there are no data that allow a direct comparison between zebrafish and other teleosts in the same procedure. It therefore remains to be seen whether the difference between zebrafish and other species is specific to the method we used in this study or extends to other learning tasks. It is important to note that the few zebrafish reaching the criterion were similar in performance to the other four species in both phase 1 and 2, reinforcing the hypothesis that the low performance of zebrafish primarily resulted from a low learning performance in this species.

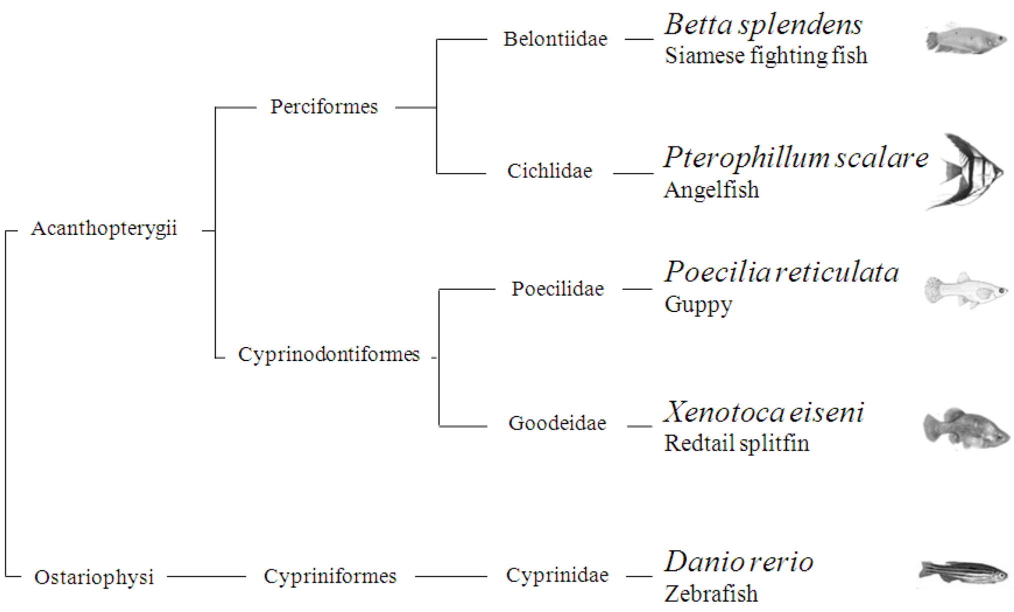
Different learning performance might in turn be explained with species-specific characteristics, such as neophobia. Consistent differences in behavior between individuals in a population, especially in the shy-bold continuum, have been reported in a variety of organisms, including many fish species (Sih et al., 2004; Dall et al., 2004); it has been termed “animal personalities” or “coping styles”. In many conditions, these different coping styles may affect the speed of acquiring a task (Amy et al., 2012; Kurvers et al., 2010; Sneddon, 2003). One might argue for instance that a shy species may have explored the experimental tank less than a bold species, thus having less time to associate the proximity to the positive stimulus with food reinforcement. However, this is not the case in our experiment, as we found that the proportion of time spent in the two choice areas by zebrafish was the same as other species.

The second difference between the species was observed in phase 1. Unlike the other four species, angelfish seem to be unable to discriminate 8 vs. 12. Such a result is puzzling and even surprising if we consider that angelfish tested with another paradigm (spontaneous shoal choice) showed the same or even better performance than mosquitofish and guppies in large number discrimination (Gómez-Laplaza & Gerlai, 2011b; Agrillo et al., 2008, 2012). *P. scalare* is larger species and, in order to match as far as possible the five species in size, we had to test sub-adult angelfish. This factor could potentially account the differences observed in this species. However we believe this is an unlikely explanation for the differences observed in this experiment, as other studies have shown that numerical abilities of very young fish are not much dissimilar from those observed in the adults (Bisazza et al., 2010). It is worth noting that, although the subjects of the five species had comparable body length, the morphological characteristics of angelfish differ from those of the other species tested: in angelfish the longitudinal axis is shortened, and the body is laterally compressed with extended dorsal and anal fins and we cannot exclude that water depth used in experimental tanks was not entirely suitable for this species.

The difficulty to understand the exact nature of angelfish peculiarity highlights one of the main problems of comparative studies: the strength of using the same methodology for testing different species may become a methodological weakness. Different species show different adaptations to their different ecological niches and, therefore, housing and testing requirements could be different in the lab; some species might be affected by such daily handling more than some others, or have perceptual or motivational characteristics that potentially render the tests more dissimilar across different species than initially planned. To assess whether the apparent inability of angelfish to discriminate the 0.67 ratio is simply an artifact of the methodology adopted, replication studies using different methods are needed (Agrillo & Miletto Petrazzini, 2012).

A third possible inter-specific difference was observed in phase 2 in which the generalization to small numbers was fully significant only for three species. The trend is, however, similar for the five species, and the likelihood ratio analyses indicated that the lack of difference between the species was 2.98 times more likely than the alternative hypothesis. One may argue that results of phase 2 might be affected by potential carry-over effects from phase 1, as all subjects performed the experiments in the same order (generalization to different numerical ratios firstly and generalization to different total numerosities secondly). However, it is worth noting that in the whole Experiment 1 fish were trained only in a 0.50 ratio and were exposed to more difficult ratios without receiving any reinforcement.

In summary, with the possible exception of the angelfish results in one of four generalization tests, this study provides scarce evidence that quantification systems differ across teleosts. There is current debate regarding pre-verbal numerical systems. Some scholars argue that they are the same in all vertebrates, inherited from a common ancestor; others believe that each species has evolved its mechanisms in relation to the constraints imposed by the nervous system and the ecological problems faced in the environment. From a phylogenetic point of view, the five species studied here are distantly related. According to recent estimates, the Ostariophysi (Fig. 24), the group to which zebrafish belongs, and the Acanthopterygii, the group which comprises the other four species, diverged more than 250 million ago (Steinke et al., 2006). They also encompass a broad spectrum of ecological adaptations. For example, some species live in open areas and others densely vegetated shallow waters, some are highly gregarious and other basically solitary, some care their young and other provide no form of parental care. The finding of so few inter-specific differences seems more in accord with the existence of ancient quantification systems inherited from a common ancestor. On the other hand, the species have been compared in a single context, and they may reveal larger differences if studied in wider spectrum of domains.



FIig. 24: *Phylogenetic relationship of the five teleost species used in the study.*

The observation made in this study that fish can easily generalize to sets of reduced numerosity but not to sets of enlarged numerosity deserves consideration. The failure to generalize the numerical rule learned in 5 vs. 10 and 6 vs. 12 in contrast to 25 vs. 50 items is particularly surprising considering that previous studies (Agrillo et al., 2010 and see Section 4.1) showed that mosquitofish can successfully generalize to even larger numerosities, such as 100 vs. 200, provided that they receive some reinforced trials on these new stimuli. One could argue that during training, fish may have learned to choose a precise numerosity instead of learning a number rule (i.e. choose the larger/smaller quantity), and later they preferred the quantity closest to that previously reinforced. For example, a fish trained to choose 12 over 6 items during 25 vs. 50 probe trials might prefer 25 items because it is the closest to the reinforced numerosity. In this case, however, we would expect the same phenomenon to occur during the generalization to smaller numerosities, which did not happen. In addition, we expect an opposite performance depending on whether fish have been trained toward the smaller or the larger numerosity. Yet, no difference was found between these two conditions, thus excluding the possibility that the direction of the training might have interfered with the direction of the variation of total set size.

We can only speculate about the meaning of this result. In nature, some items in a set may partially hide each other or temporarily disappear behind objects, thus reducing the visible total set size even if their composition is constant. For example, during the comparison of 5 vs. 10 conspecifics, fish might be required to continue the enumerating process when the perceived numerosity is reduced, for example when only 4 vs. 8 fish are visible. In this sense, the cognitive systems of these species might have been selected to generalize the numerical rule to another contrast with a reduced total set size. In contrast, it is physically implausible that groups of objects increase their numerosity without altering their composition. In other words, while 2 vs. 4 would appear as another version of the 5 vs.10 task, the shift from 5 vs. 10 to 25 vs. 50 items might appear to fish as a novel task, preventing generalization of the same numerical rule from smaller to larger numbers. It will be a challenging task to determine whether other vertebrate species show the same generalization pattern.

As a last remark, we would like to note one important implication of the findings from Experiment 2. While the results of the training phase in Experiment 1 would superficially suggest cross-species differences in numerical abilities, the difference observed between redtail splitfin and zebrafish in another type of discrimination showed the true nature of zebrafish low performance. When investigating the existence of differences between experimental groups in one cognitive domain, it is always important to include control tests done in other domains to exclude the possibility that the observed differences depend on concomitant factors, such as personality, motivation, or attention differences. This is routinely performed in other disciplines (i.e., cognitive psychology), but still rarely adopted in comparative psychology studies.

5. FACTORS AFFECTING NUMERICAL ABILITIES IN FISH

In natural environments there are several ecological factors that can affect animal behavior and play a key role in decision making. This Section describes the salience of two factors that can influence quantity discrimination abilities in fish: cooperative behavior and the perception of items in motion.

5.1. Collective enhancement of numerical acuity by meritocratic leadership in fish

We know that groups of animals are capable of marvelous feats of collective action that emerge from aggregation of numerous small contributions from a large number of individuals often through elegantly simple local mechanisms (Couzin, 2009). In addition, it has been recognize that collective behavior allows grouping animals to solve problems that go beyond the capacity of the single individuals in a way that cannot be implemented by singletons. For example bird flocks orient better than single individuals: flocks of pigeons (3-6 individuals) are less scattered around the target migration destination and have a shorter homing time in comparison with single pigeons (Tamm, 1980). Three-spine stickleback fish make more accurate decisions, avoiding a potential predator (Ward et al., 2008) or choosing the more attractive replica of a conspecific (Sumpter et al., 2008), as group size increases. Interestingly, in human literature several studies have provided evidence that groups perform better than individuals on a range of problem-solving tasks (Krause et al., 2010, Laughlin et al., 2002, Kerr & Tindale, 2004).

To explain better performance of groups in humans and animals two models of collective decision making have been proposed. The first model refers to the so-called “many wrongs” principle (from here called MW) (Galton, 1907). When each individual makes an estimate that is a close approximation to the correct one but with some error, if these errors are randomly distributed around the true mean, they will cancel each other and the whole crowd will be more accurate than most if not all its single members. The MW principle has been suggested as an explanation for the advantage of group navigation in birds (Simons, 2004). This mechanism depends on large numbers of individuals in the crowd to be effective. For dyads, it predicts that the group accuracy will be the average of its members.

This model assumes that all individuals have access to the same information. However, information is not often equally distributed and individuals differ in their informational status. In these circumstances a second model might explain this phenomenon. Such model, might be referred as "Meritocratic Leadership" (ML) and applies if some members are more accurate than others to accomplish the task. In this scenario the group would enjoy an advantage provided that collective decisions are guided by its best performing members. This mechanism is thought to be at the basis of collective decision in honeybees where few informed individuals can bias the decision of the whole group (Seeley et al., 1991). Bees have evolved specific recruit signals that help informed individuals to guide their conspecifics. However, in some animal groups crowding can limit individual recognition and the possibility to detect each other: in this condition it is difficult for an uniformed individual to recognize the informed ones

Nevertheless, different computational models have been proposed that demonstrate how a group may, for example, forage or navigate accurately without signaling between group members or without group members needing to recognize who the best informed individual is (Counzin et al., 2005). Such models of leadership and collective actions in animals are mute when it comes to the most basic unit of social interaction: a dyad. Whether pre-social animals such as fish can even engage in any useful one-to-one collaborative effort is unknown and not accounted for by existing theories of collective action in animals.

To address this question, we compared the performance of individual and dyads of guppies (*Poecilia reticulata*) in two different numerosity discrimination tasks. Guppies, and other small fish that risk predation, can discriminate numerosities and use this ability to reduce the risk by spontaneously joining the larger shoal of conspecifics (Agrillo et al., 2008a, 2012, Dadda et al., 2009). Furthermore, they can also learn to select the more or less numerous display of abstract objects even when controlled for other confounds of magnitude such as size and density (Agrillo et al., 2009, 2010). Typically, guppies and other small fish can readily discriminate up to 1:2 ratio when numerosities larger than 4 are involved, although they exhibit a better numerical acuity within a limited range of numerosities (≤ 4) (Agrillo et al., 2008a; 2012). However, it is not known whether two guppies schooling together would be better at numerosity discrimination than they would as individuals.

5.1.1. Experiment 1: Spontaneous discrimination of shoals differing in numerosity

In Experiment 1, we used a shoal choice test to compare the accuracy of female guppies tested individually with that of female guppies tested in dyads in a shoal size discrimination. To investigate the role of familiarity in dyads, half of the dyads were composed of familiar individuals (fish that had lived in the same tank for at least 20 days), and half of non-familiar individuals. Singletons and dyads were presented the numerical contrast 4 vs. 6, just above the threshold ratio reported for fishes in experiments using this procedure (Agrillo et al., 2008a, 2012).

Subjects

Adult female guppies (*Poecilia reticulata*) were stocked at the Laboratory of Comparative Psychology (University of Padova) and maintained for one month in 150 one-stock aquaria containing mixed-sex groups (15 individuals with approximately a 1:1 sex ratio). Aquaria were provided with natural gravel, an air filter, and live plants. Both stock aquaria and experimental tanks were maintained at a constant temperature of 25 ± 1°C and a 14:10 h light : dark (L:D) photoperiod with an 18-W fluorescent light. Before the experiment, fish were fed twice daily to satiation with commercial food flakes and live brine shrimp (*Artemia salina*).

We tested a total of 150 subjects (ranging from 3 to 6 cm in length). Forty-six subjects were singly tested, while 104 fish were tested in pairs (n = 52). In order to investigate the potential role of familiarity, half of the pairs (n = 26) was composed by familiar individuals (fish living in the same tank for at least 20 days), while the other half was composed by non-familiar fish.

Apparatus

The experimental apparatus was one previously used to study numerical competence in adult guppies (Agrillo et al., 2012) and was composed of three adjacent tanks (Fig. 25). The central one, called “subject tank”, was 60 x 36 x 35 cm. At the two ends, facing the subject tank, there were two “stimulus tanks” (10 x 36 x 35 cm) into which two shoals differing in numerosity were placed. Water level was equal to 10 cm and the walls were covered with green plastic to prevent subjects from seeing outside. Each tank was lit by one fluorescent lamp with water maintained at a temperature of 25° ± 2°C. A video camera was suspended about 1 m above the test tank to record the position of the subjects during the tests.

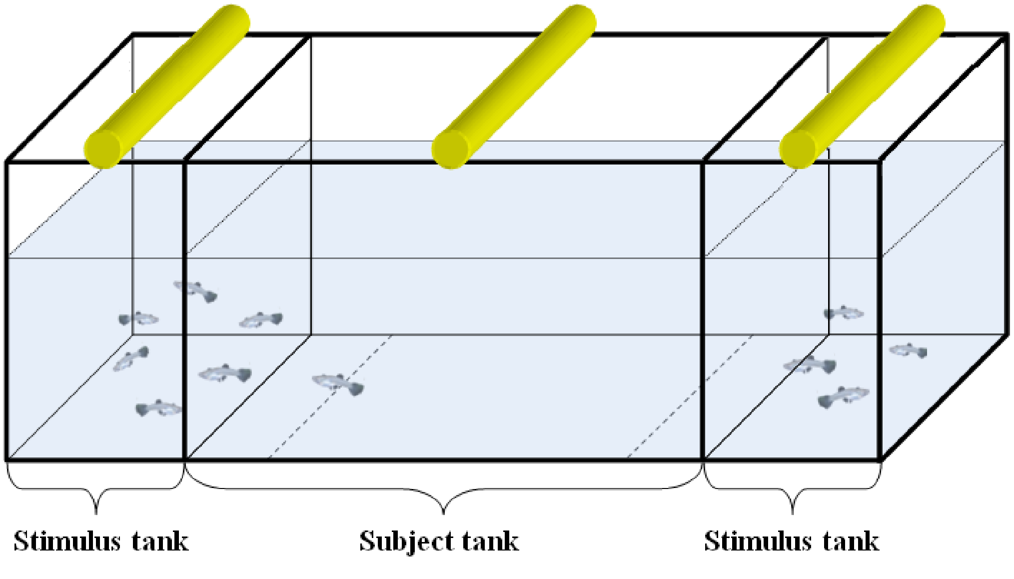


Figure 25: *Schematic representation of the experimental apparatus: Subjects were inserted in the middle of three adjacent tanks. Two groups of social companions differing in numerosity (4 vs. 6) were presented at the two bottoms and the proportion of time spent near the larger shoal was taken as a measure of their numerical acuity. Dotted lines show the preference area.*

Procedure

As said in Section 1.5, single fish are known to re-join the largest group of social companions when placed in an unknown environment.

Fish were presented with the same numerical contrast (4 vs. 6) and tested in two different conditions: singletons vs. dyads. In the single condition the subject was introduced into a hollow cylinder in the center of the subject tank. After 2 min the cylinder was carefully raised up and subject was allowed to acclimate for 2 min. After this period the subject was observed for 15 min. Shoal preference was calculated as the proportion of time spent by the subject shoaling within a distance of 11 cm from the glass facing the stimulus tanks (preference area). Subjects that did not visit each stimulus sector at least three times were discarded.

As regard fish tested in dyads, the experimental procedure was the same. Two subjects were simultaneously inserted in the subject tank and their behavior was observed for 15 min. The shoal preference was calculated as the time spent by both subjects in the preference area. In the event that subjects were in the opposite preference area, or that only one subject was in a preference area, the choice of fish was not included in the analysis. Mean ± standard deviations were provided. Statistical tests were carried out using SPSS 18.0.

To further verify which model (MW or ML) better explain the performance of the dyads in our experiments, we generated two sets of “simulated dyads”: we sampled the data from individually tested fish by randomly selecting and assigning two individuals to a dyad. For one set of simulated dyads, we calculated the average accuracy of the two members as the dyadic accuracy and for the other set we assigned the more accurate member’s accuracy to the simulated dyad.

Results

Two singletons and two dyads were discarded from the analysis because did not visit each stimulus sector at least three times. As no difference between familiar pairs and non-familiar pairs was found (mean ± SD respectively: 0.592 ± 0.243 and 0.575 ±

0.197; independent *t*-test: *t*(48) = 0.324, *p* = 0.747), the two groups were pooled together in the main analysis.

The larger shoal was significantly preferred by dyads (one sample *t*-test, *t*(49) = 2.741, *p* = 0.009) but not by individually tested fish (*t*(43) = 0.128, *p* = 0.898) that spent an equal amount of time with the two shoals (Fig. 26).

Dyads spent significantly larger proportion of their time with the larger shoal than did the single fish (independent *t*-test, *t*(92) = 1.727, *p* = 0.043).

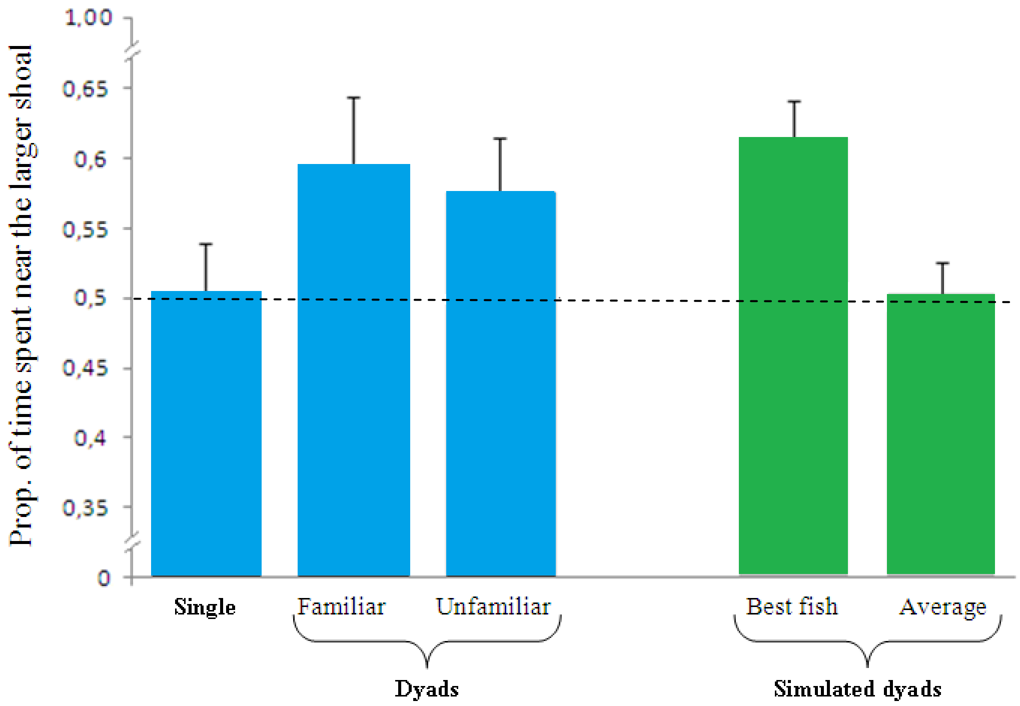


Fig. 26:.*Dyads were significantly better than single fish. Real dyads were also more accurate than the "average of two" simulated data set but did not differ from the "best of two" simulated data set.*

Real dyads were more accurate than the “average of two” simulated data set (*t*(98) = 2.136, *p* = 0.035) but did not differ from the “best of two” simulated data set (*t*(98) = 0.730, *p* = 0.467) thus providing evidence against the MW hypothesis. This conclusion is supported by Bayesian model selection procedure. Bayes factor (Penny et al., 2004) computed on BIC (Bayesian information criterion) show that that ML model is 4.64 times more likely than MW model to explain the performance of the dyads in our experiment (conventionally there is a evidence for a model against an alternative when this value is greater than 3: Raftery, 1995).

5.1.2. Experiment 2: Trained abilities to discriminate between sets of stimuli differing in numerosities

Experiment 1 demonstrated that dyadic interaction enhances numerosity discrimination in a directionally specific (i.e. choose larger quantity), socially and ecologically relevant context. One may argue that these specific features may render the collective benefit observed in Experiment 1 extremely specific. In addition in the previous experiment, stimuli were not controlled for continuous quantities. In order to search for a more general evidence for dyadic collective benefit, in Experiment 2, adult male guppies were trained to discriminate between two set of inanimate objects and were tested singly or in dyads in discriminations of increasing difficulty (8 vs. 12 and 9 vs. 12). The 2:3 ratio corresponds to the upper limit in the ability of fish to discriminate quantities beyond 4 and 3:4 is just above this threshold (Agrillo et al., 2010 and see Section, 4.1)

Subjects

Subjects were 50 adult male guppies. Before the experiment, fish were maintained in the same conditions described for Experiment 1.

Apparatus and stimuli

We used the apparatus previously described (see Section 4.1) to study numerical competence in fish.

Stimuli were groups of black dots differing in size on a white background. Different numerical contrasts were presented: 5 vs. 10 and 6 vs. 12 (1:2 ratio) in the training phase; 8 vs. 12, and 9 vs. 12 (2:3, and 3:4 ratios, respectively) in the test phase. Stimuli selected for the experiment were extracted from a pool of 24 different pairs for each numerical contrast. The size and position of the dots were changed across sets. Stimuli were controlled for continuous quantities using the procedure described in Section 4.1.

Eight identical experimental tanks were used. They were placed close to each other on the same table and lit by two fluorescent lamps (36 W). A video camera was suspended about 1 m above the experimental tanks and used to record the position of the subjects during the tests.

Procedure

The experiment was divided into three different phases: individual training, test, and control of individual performance of dyads. During the training phase, we presented an easy numerical ratio (1:2) with the purpose of training the fish to the new task and selecting those fish successfully accomplished the task.

In the test phase, we assessed whether fish accuracy to discriminate novel numerical ratios (2:3 and 3:4) varied when subjects were tested singly or in dyads.

In the control of individual performance of dyads, subjects previously tested in dyads were observed individually in their capacity to discriminate 2:3 and 3:4 numerical ratio, in order to assess whether the performance of dyads might be explained by a simultaneous increase in accuracy of both individuals, or by the presence of only one individual having a better performance.

Individual training

The individual training followed the procedure previously describe in Section 4.1. It consisted in 12 reinforced trials equally subdivided in three consecutive (days 1 to 3) days and 4 probe trials in the following two days (days 4-5).

Only fish who met the learning criterion (defined as 60% of the time spent near the reinforced numerosity in probe trials) were selected for the test phase.

Test

After two-day interval (in which fish received a total of four reinforced trials, two each day), fish were divided in two groups: singletons vs. dyads. Fish included in the former group were observed individually; fish included in the latter group were tested in pairs. Dyads were assembled each morning, one hour before the beginning of the test;

only in the evening (after the test) each fish was inserted singly in the tank. Each pair was always composed by the same individuals.

Three probe trials were presented each day for four consecutive days (days 8 to 11). Fish were presented with two novel numerical ratios, 2:3 (8 vs. 12), and 3:4 (9 vs. 12), and six presentations for each ratio in a pseudo-random sequence. The inter-trial interval lasted three hours. Two reinforced trials presenting the numerical contrasts of the training (5 vs. 10 and 6 vs. 12) were alternated in the probe trials. The proportion of time spent in the ‘choice areas’ during probe trials was used as a dependent variable. As Experiment 1, time spent in the choice area in the dyad condition was considered only when both subjects were simultaneously in the same choice area. Mean ± standard deviations were provided. Statistical tests were carried out using SPSS 18.0.

Control of individual performance of dyads

On day 12, fish tested in pairs were separated and observed individually in their ability to discriminate 2:3 and 3:4 numerical ratio. Four probe trials were presented, two each numerical ratio; two reinforced trials presenting the numerical contrasts of the training (5 vs. 10 and 6 vs. 12) were alternated in the probe trials.

The comparison between dyads, average of two individuals and best individuals was calculated on the average accuracy of 2:3 and 3:4 ratio.

Results

Thirty fish out of 50 reached the criterion and hence started the test phase. Ten subjects were included in the singleton condition, 20 in the dyad condition.

As no difference in the accuracy was reported between fish trained with the larger numerosity (mean ± SD: 0.648 ± 0.118) and those trained with the smaller numerosity as positive (0.564 ± 0.069, independent *t*-test $t(18) = 1.98$, $p = 0.063$), the two groups were pooled together in the main analyses.

Dyads did significantly better than singletons (ANOVA, $F(1,18) = 6.492$, $p = 0.020$) and there was a significant effect of numerosity ratio ($F(1,18) = 10.282$, $p = 0.005$; Interaction $F(1,18) = 0.011$, $p = 0.917$). Discrimination was above chance for 2:3 ratio both by singletons (one sample *t*-test, $t(9) = 2.782$, $p = 0.021$) and by dyads ($t(9) = 5.920$, $p < 0.001$), while the 3:4 ratio was only discriminated better than chance by dyads ($t(9) = 2.459$, $p = 0.036$) but not by singletons ($t(9) = 0.422$, $p = 0.683$, Fig. 27).

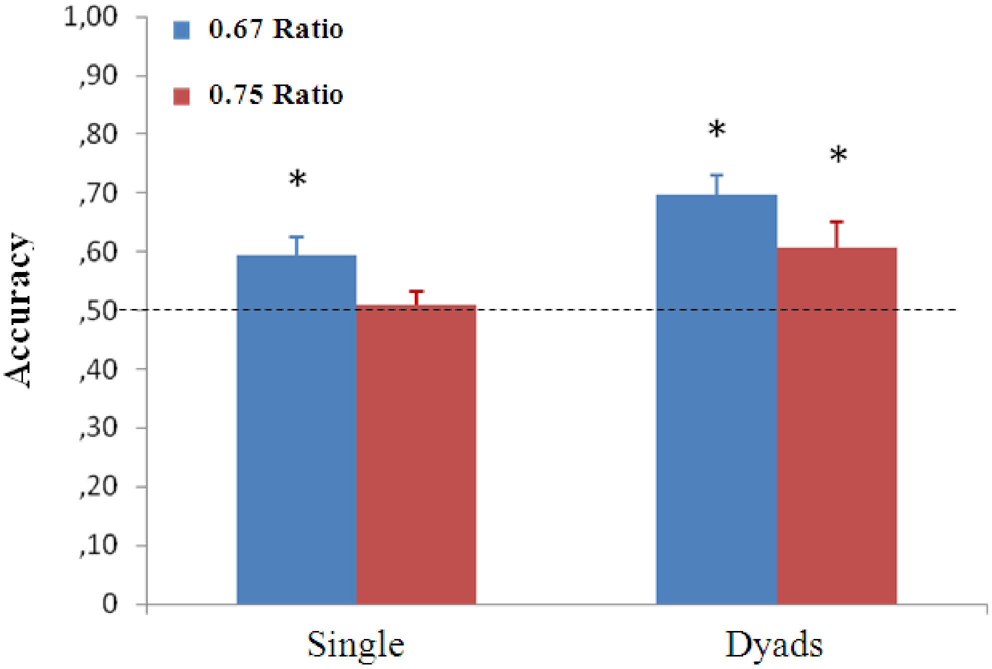


Fig. 27: *Results of the test phase. Accuracy is plotted against numerical ratio, for single fish and dyads. Results parallels those reported in Experiment 1, with dyads showing a better numerical acuity than those reported in single fish. Asterisks denote a significant departure from chance level (P<0.05). Bars represent the standard error.*

When analyzing the individual performance of the dyads, it was found that the accuracy of the dyad was superior to the average accuracy of the two members when performing individually (repeated measures ANOVA $F(2, 18) = 5.65$, $p = 0.012$; pos-hoc LSD $p = 0.045$) but did not differ from that of the best member ($p = 0.95$). The Bayes factor indicates that the ML model is 5.57 times more likely than MW model.

5.1.3. Discussion

The aim of the present study was to assess whether the performance of fish in dyad outperform that of single fish. To this purpose, two experiments were set up. In the former, we observed numerical acuity in a task requiring to discriminate between social companions. In the latter fish were trained to discriminate between sets of inanimate objects. In both experiments dyads achieved a superior level of numerical acuity. Indeed, in Experiment 1, 4 vs. 6 was discriminated by dyads but not by singletons; in Experiment 2 dyads proved able to discriminate 9 vs. 12, a discrimination not shown by singletons. As the second experiment deployed a learning paradigm with stimuli carefully controlled for continuous quantities these results are likely to reflect that dyad outperform the singletons in a task in which only numerical information could be available.

Numerosity discrimination has been repeatedly demonstrated in many animal species including invertebrates (Gross et al., 2009), amphibians and mammals (Agrillo & Beran, 2013), as well as primates and fish. It has been shown to be beneficial in several ways. For example, bees enumerate the number of landmarks encountered during flight to relocate a food source (Dacke et al., 2008); fish use it to select the larger, safer shoal (Agrillo et al., 2008a); lions use to decide whether to fight or to flee (McComb et al., 1994). However, in this study we found that guppies tested singly show the same average discrimination limit observed in other teleost species in a free shoal choice (i.e., a 1:2 ratio; Agrillo et al., 2008a, 2012), though singleton guppies can be successfully trained to manage a 2:3 ratio (see Section 4.2). However when tested in pairs they were able to discriminate numerosities with a 3:4 ratio, a numerical acuity that mammals (Tomonaga, 2008, Beran, 2006) and birds (Emmerton & Delius, 1993) exhibit only after extensive training.

Together, the results of Experiments 1 and 2 clearly reject the MW hypothesis as the mechanism underlying collective benefit at the dyadic level (Simons, 2004). The critical point that renders this mechanism inadequate at explaining these results is the dyadic nature of collective decisions studied here. The larger the number of agents in the collective the more successful this mechanism is expected to be. With only two agents involved in collective decisions, this model predicts that the dyadic performance will be determined by average member accuracy which was not the case in both experiments. The results are consistent with the idea that dyad performance is determined by the better member taking a leadership role rather than by the “average of two”. We know that leadership can emerge spontaneously in the shoaling behavior of teleost fish (Reebs, 2000) but, once again, this type of emergent phenomenon also depends critically on group size and the computational models explaining effective leadership (Couzin et al., 2005) have not been tested in group sizes of $N < 10$. Indeed, social learning in guppies mediates meritocratic leadership where younger shoal mates learn to follow older or more successful foragers (Lachlan et al., 1998, Laland & Williams, 1997). Exactly how the pairs of guppies tested here could assign the leadership role among them is not possible to tell from our data presented here.

By verbally exchanging decision confidences, human decision makers with similar competence levels achieve a collective benefit over and above their best individual (Bahrami et al., 2010). For example, it has been observed that dyadic performance was superior to individual in an approximate enumeration task, irrespective of the familiarity between the dyad members (Bahrami et al., 2013). Interestingly the level of familiarity does not affect performance in fish too, but whether the behavior of guppy dyads implies social means of signal sharing such as confidence is unknown and beyond the scope of this study. Computationally, the confidence sharing model (Bahrami et al., 2010) does not offer a fixed prediction for dyadic collective benefit but instead suggests that collective benefit is proportional to similarity of competence between dyad members. But accurate assessment of similarity requires far more test trials than we could have administered here rendering it impossible to formally test the predictions of the confidence sharing model. Whether special physical and/or social cues are employed in determining the dyadic leader is an intriguing and important question for future research. The counter intuitive fact, now beyond doubt, is that collaboration among presocial animals is observable with minimum possible social group size of two agents.

5.2. Numerical acuity of fish is improved in the presence of moving targets, but only in the subitizing range*

** This research is a modified version of "Agrillo, C., Miletto Petrazzini E. M., & Bisazza, A. (2013). Animal cognition, 1-10".*

Several studies in cognitive psychology have demonstrated that humans use three types of processes—counting, estimating, and subitizing—to establish which group of items is more numerous (Chesney & Haladjian, 2011). Counting (Gelman & Gallistel, 1978) assesses numerosity by assigning each item an exact number label from an ordered count list. This process, uniquely human, is very accurate but relatively slow, with response time becoming longer as the number of items in the set increases. Estimating (Kaufman et al., 1949) assesses the numerosity of a set quickly, instead, without a serial count. This process is supposed to be supported by an approximate number system (ANS) that is fast but poorly accurate (Nieder & Dehaene, 2009). Subitizing (Jevons, 1871; Revkin et al., 2008) is another way to estimate the numerosity of a set without a serial count. This process is fast and accurate but limited to up to 3–4 items. While for numbers greater than 4 discrimination between two quantities is dependent on their ratio the numerical ratio is usually irrelevant in the range of 1–4 Several authors hypothesize that subitizing is based on a system for representing and tracking up to 4 objects in parallel (Chesney & Haladjian 2011; Feigenson et al., 2004; Trick & Pylyshyn, 1994), known as the object tracking system (OTS).

Other authors, however, argue that it is not necessary to invoke two distinct numerical systems and that ANS suffices to account for discrimination in the whole numerical range (Gallistel & Gelman, 1992; van Oeffelen & Vos, 1982; Vetter et al., 2008). As noted by Gallistel and Gelman (1992), the representation of larger numbers is more variable and, as a consequence, representation of nearest values may overlap in the large number range, leading to lower accuracy. In contrast, in the subitizing range (1–4), ANS representations would have lower variability and there would be almost no overlap; thus, correct values would be consistently produced, leading to very accurate performance. In addition, while adults can discriminate even a 0.9 ratio (Halberda et al., 2008), the most difficult ratio in the subitizing range is 0.75 (3 vs. 4 items); therefore, ratio dependence might not appear, due to a ceiling effect in performance (Ross, 2003). Others have suggested that the different performance results in the subitizing range might be due to pattern recognition rather than to numerical processes per se. In two-dimensional displays, small sets of items are typically arranged in recognizable geometric patterns (i.e., 1 item = a dot; 2 items = a line; 3 items = a triangle), which is not possible for sets of more than 4 items (Mandler & Shebo, 1982; Neisser, 1967; Woodworth & Schlosberg, 1954).

One way to test the above-mentioned alternatives is by looking at factors other than ratio dependency. For example a potential prediction of the one-system hypothesis is that manipulation of physical properties of the stimuli should never have opposed effects on the estimation of small and large numbers, while the hypothesis for the existence of separate systems would allow for this possibility. Trick (2008) tested whether item heterogeneity affects performance differently in the subitizing and estimation ranges in adult humans. The results showed that heterogeneity slowed enumeration in the subitizing range and sped-up enumeration in the estimation range, a dissociation that would seem to negate the one-system hypothesis. Moving versus static items represent another variable that seems to affect numerical estimation differentially in humans. Trick and colleagues (2003) observed that even very slow motion reduced enumeration speed for stimuli containing 6–9 items, while the enumeration of 1–4 items was not affected when items were in motion. Similarly, Alston and Humphreys (2004) presented static and moving items, finding that faster and more accurate enumeration occurred in the subitizing range given the presence of moving items. Again these results tend to support the existence of separate systems for small and large-number enumeration.

In recent years, numerical abilities have been reported in numerous animal species. Comparative psychology is explicitly focused on those processes that do not require symbolic number labels (estimation and subitizing). The similar performance reported in the literature for human and non-human species has led several authors to hypothesize the existence of the same numerical abilities among vertebrates (Beran, 2008a; Feigenson et al., 2004). As in cognitive psychology, there is an open debate as to whether non-human animals display a single ANS for discrimination over the whole numerical range (Evans et al., 2009; Perdue et al., 2012; Ward & Smuts, 2007), or a distinct OTS over the small-number range (Bonanni et al., 2011; Cutini & Bonato, 2012; Hauser et al., 2000; Hunt et al., 2008). To date, empirical studies have focused on comparing ratio dependency on accuracy and reaction times in small- and large-number discrimination. For instance, Agrillo et al. (2012) compared the performance of humans and guppies, presenting the same ratios for small (≤ 4) and large (≥ 4) quantities and finding that accuracy for both species was affected by the ratio in the large- but not in the small-number range. Yet these results are subject to the same criticism raised for human studies, and different ratio sensitivity in the range of 1–4 does not automatically imply the existence of two distinct numerical systems (Gallistel & Gelman, 1992; Ross, 2003).

In the present study, we adopted a different approach to this issue. We tested whether, as in humans, the motion of the items for enumeration had a different influence on large- and small number discrimination in fish. For this purpose, we trained guppies to discriminate between groups containing different quantities of two-dimensional geometric figures, using either static or moving objects.

Subjects

Twelve adult guppies (*Poecilia reticulata*) were tested. Subjects were maintained at the Department of General Psychology in 150 one-stock aquaria containing mixed-sex groups (15 individuals with approximately a 1:1 sex ratio). Aquaria were provided with natural gravel, an air filter, and live plants. Both stock aquaria and experimental tanks were maintained at a constant temperature of 25 ± 1 °C and a 14:10 h light:dark (L:D) photoperiod with an 18 W fluorescent light. Before the experiment, fish were fed twice daily to satiation with commercial food flakes and live brine shrimps (*Artemia. salina*).

Apparatus and stimuli

The experimental apparatus was similar to the one used in previous experiments (see Section 4.1), with the exception that at the two short ends of the tank, two identical monitors (19 inch) were used to present the stimuli (Fig. 28).

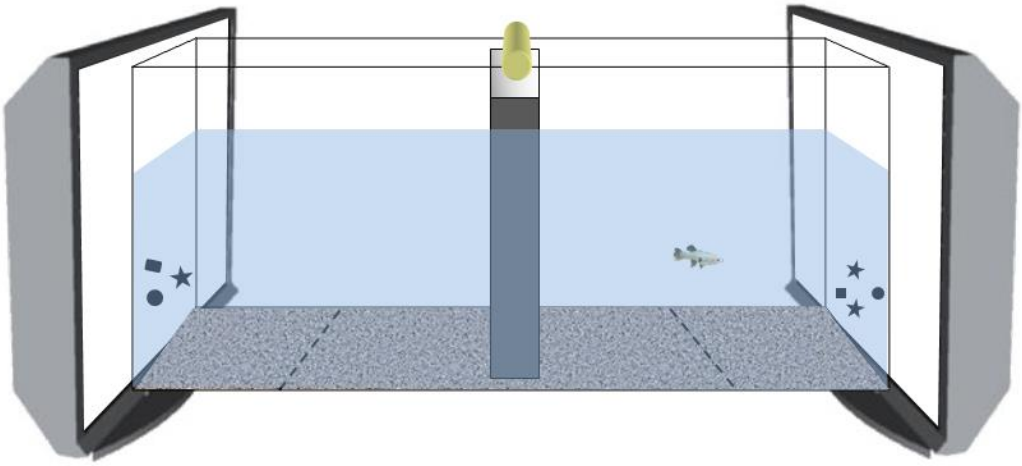


Fig. 28: *Experimental apparatus. Subjects were housed in an experimental tank for the duration of the experiment. Stimuli were presented at the two ends of the tank using two PC monitors.*

The stimuli employed in the training phase were pairs of geometrical figures (13 x 13 cm each array) differing in numerosity: 2 versus 4 (small-number range) and 6 versus 12 (large-number range). In the test phase, we presented the following numerical contrast: 3 versus 4 (small-number range) and 9 versus 12 (large-number range). Each array could contain circles, ovals, triangles, crosses, stars, squares, and rectangles (black figures on a white background). Stimuli were controlled for continuous quantities using the procedure described in Section 4.1.

For each numerical contrast, we set up two different conditions: in the first (‘moving stimuli’), the items were moving on the screen; in the second, the same figures were presented without any motion (‘static stimuli’). As the overall quantity of motion could be another non-numerical cue used by animals to discriminate between two numerosities (Agrillo et al., 2008a; Krusche et al., 2010; Gòmez-Laplaza & Gerlai, 2012), two different sub-conditions were created with the moving stimuli. In the first sub-condition (controlled motion), the overall quantity of motion was equated between the larger and the smaller group. For instance in the 2 versus 4 contrast, the figures included in the smaller group moved twice as fast as those included in the larger group. However, a by-product of controlling for total motion was that faster-than-average items would appear more frequently in the smaller group, and fish might use this cue instead of number. As a consequence, we set up another sub-condition (non-controlled motion) where number and quantity of motion were simultaneously congruent. In this case, in the 2 versus 4 contrast, the quantity of motion for the larger group was double what it was for the smaller group. For each numerical contrast, half of the stimuli were controlled for motion, and the other half were not.

Moving and static stimuli were created and presented using Adobe Flash CS4®. For each numerical contrast, a total of 20 different pairs of stimuli were employed and alternated in pseudo-random order.

Six identical experimental tanks were employed. They were placed close to each other, on the same table, and lit by two fluorescent lamps (36W). A video camera was suspended about 1 meter above the experimental tanks and used to record the position of the subjects during the tests.

Procedure

The experiment was divided into three different steps: pre-training, training, and test. Pre-training was set up to familiarize the fish with the experimental apparatus. Subsequently, all fish were singly trained to discriminate an easy numerical ratio (0.50), both within (2 vs. 4) and outside (6 vs. 12) the subitizing range: half of the fish were tested in the presence of moving stimuli; the other half was tested in the presence of static stimuli. In the test phase, we assessed whether they could generalize the numerical rule to a more difficult numerical ratio in both
the small- (3 vs. 4) and the large-number (9 vs. 12) ranges.

Pre-training

To familiarize fish with the experimental environment, we allowed a two-day habituation period, starting 10 days before the beginning of the experiment. On the first day of habituation, a group of 6 fish was introduced to a slightly larger version of the tank (60 x 40 x 35 cm); on the second day, fish were grouped in three pairs and each pair was introduced to one of three experimental tanks (6 h each day). On both days, subjects were exposed to continuous changes in the background colour of the monitors (alternating between black and white).

After a five-day interval, when they were kept undisturbed in their home tank, we administered another identical two-day habituation treatment. The day before the beginning of the experiment, each fish was individually housed in one of the six experimental tanks.

Training

Fish were randomly assigned to one of two conditions: half of the fish were trained in the presence of moving stimuli; the other half in the presence of static stimuli. During this phase, they were subjected to four trials per day (three consecutive days, for a total of 12 trials). Soon after the stimuli appeared on the screens, the experimenter used a Pasteur pipette to release the food reward (*Artemia* nauplii) in correspondence with the reinforced numerosity; an identical syringe was used to simultaneously insert pure water close to the opposite stimulus. Subjects were left free to feed for 7 min. After this time, stimuli disappeared from the screen. The inter-trial interval lasted 3 h. Stimuli were presented in a pseudo-random sequence and the left–right positions of the stimuli were counterbalanced over the course of the trials. For each condition, half of the subjects were trained to move toward the larger numbers, whereas the other half was trained toward the smaller numbers as positive.

In order to measure learning to discriminate between reinforced and non-reinforced numerosity, on days 4 and 5, two probe trials were alternated each day with two reinforced trials (four overall probe trials for both days). In the probe trials, stimuli appeared on the screen for 4 min; no reinforcement was provided (extinction procedure) and the time spent by fish within a 12-cm distance (‘choice area’) from the monitors showing the stimuli was recorded as a measure of their capacity to discriminate between the two numerosities. Reinforced trials were identical to those described for days 1 to 3. To avoid the possibility of fish using local/spatial cues in their tank, each subject was moved from one tank to another at the end of each day.

Test

After a two-day interval, three probe trials were presented each day for four consecutive days (days 8–11). Novel numerical contrasts with higher ratios were presented at this time: 3 versus 4 (within the subitizing range) and 9 versus 12 (large-number range). Fish previously trained with moving stimuli were presented with moving stimuli; fish trained with static stimuli were shown static stimuli. The inter-trial interval lasted 3 h. Two reinforced trials with the same stimuli as presented in the training phase were alternated with the probe trials. As a dependent variable, we considered the proportion of time spent in the ‘choice areas’ during probe trials. Proportions were arcsine square-root transformed (Sokal & Rohlf, 1995). Mean ± standard deviations were provided. Statistical tests were carried out using SPSS 18.0.

Results

Training

Moving stimuli

Fish spent significantly more time near the reinforced numerosity in both the 2 versus 4 (one sample *t*-test, $t(5) = 6.184$, $p = 0.002$) and the 6 versus 12 (one sample *t*-test, $t(5) = 2.655$, $p = 0.045$, Fig. 29) contrasts. No difference between the two numerical contrasts was found (paired *t*-test, $t(5) = 0.535$, $p = 0.616$). When we contrasted the controlled and non-controlled motion condition, we found no difference for either 2 versus 4 (controlled motion, mean ± SD: 0.730 ± 0.302; non-controlled motion: 0.762 ± 0.217; paired *t*-test, $t(5) = 0.123$, $p = 0.907$) or 6 versus 12 (controlled motion: 0.655 ± 0.258; non-controlled motion: 0.833 ± 0.258; paired *t*-test, $t(5) = 1.332$, $p = 0.240$).

On the whole, we found no difference in accuracy between fish reinforced with the larger or smaller numerosities as positive (larger numbers as positive: 0.708 ± 0.105; smaller numbers as positive, 0.809 ± 0.128; independent *t*-test, $t(4) = 1.052$, $p = 0.352$).

Static stimuli

Fish spent significantly more time near the reinforced numerosity in 2 versus 4 (one sample *t*-test, *t*(5) = 2.643, *p* = 0.046) and 6 versus 12 (one sample *t*-test, *t*(5) = 4.752, *p* = 0.005, Fig. 29). No difference between the two numerical contrasts was found (paired *t*-test, *t*(5) = 0.702, *p* = 0.514). On the whole we found no difference in the accuracy between fish trained with the larger or smaller numerosities as positive (larger numbers as positive, 0.779 ± 0.083; smaller numbers as positive, 0.639 ±0.054; independent *t*-test, *t*(4) = 2.443, *p* = 0.071).We also found no difference in accuracy between trials controlled for density (0.651 ± 0.329) and those controlled for overall space (0.744 ± 0.195, paired *t*-test, *t*(5) = 0.405, *p* = 0.702).

When we compared the two conditions (moving vs. static stimuli), we did not find any difference between fish trained with moving stimuli and those trained with static stimuli (2 vs. 4: independent *t*-test, *t*(10) = 0.885, *p* = 0.397; 6 vs. 12: *t*(10) = 0.610, *p* = 0.556).

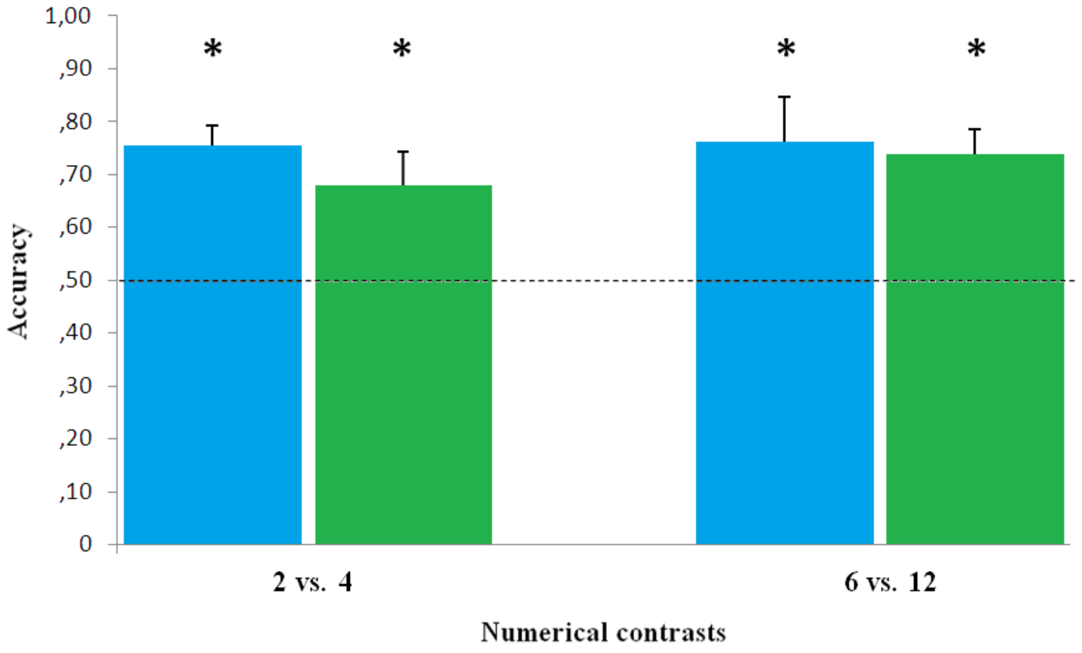


Fig. 29: *Results of the training phase. Accuracy (proportion of time spent near the reinforced numerosity) is plotted against numerical contrasts, for moving (blue columns) and static (green columns) stimuli. Fish learned to discriminate the two numerical contrasts, with both static and moving stimuli. Asterisks denote a significant departure from chance level (P<0.05). Bars represent the standard error.*

Test

To assess whether the ability to discriminate a 0.75 ratio varies as a function of movement and the numerical range, we performed a repeated measures ANOVA with Condition (Moving/Static stimuli) as the between subjects factor and Numerical contrast (3 vs. 4/9 vs. 12) as the within subjects factor. Main effects of Condition ($F(1, 10) = 10.668$, $p = 0.008$) and Numerical contrast ($F(1, 10) = 5.460$, $p = 0.042$) were found. The interaction was also significant ($F(1, 10) = 5.054$, $p = 0.048$), suggesting that fish succeeded with items in motion only in the small number range. Because of the significant interaction, in subsequent analyses we examined the effect of moving and static stimuli separately.

Moving stimuli

Fish spent more time near the reinforced numerosity in the 3 versus 4 contrast (one sample *t*-test, $t(5) = 5.639$, $p = 0.002$), while no significant discrimination was found in the 9 versus 12 contrast ($t(5) = 0.363$, $p = 0.731$, (Fig. 30). Paired *t*-tests showed a significant difference between the two numerical contrasts ($t(5) = 2.655$, $p = 0.045$). When we contrasted the controlled and the non-controlled motion conditions, we found no difference for 3 versus 4 (controlled motion: 0.843 ± 0.100; non-controlled motion: 0.770 ± 0.123; paired *t*-test, $t(5) = 1.550$, $p = 0.182$), while we did find a significant difference for 9 versus 12 (controlled motion: 0.396 ± 0.155; non-controlled motion: 0.623 ± 0.291; $t(5) = 2.733$, $p = 0.041$).

Static stimuli

Fish did not discriminate either 3 versus 4 (one sample *t*-test, $t(5) = 0.089$, $p = 0.933$) or 9 versus 12 ($t(5) = 0.251$, $p = 0.812$, Fig. 30). Paired *t*-tests showed no difference between the two numerical contrasts ($t(5) = 0.054$, $p = 0.959$).

Lastly, we analyzed whether the overall accuracy differed between moving and static stimuli for each numerical contrast. Independent *t*-tests showed that fish were more accurate in the presence of moving stimuli for 3 versus 4 ($t(10) = 3.511$, $p = 0.006$) but not 9 versus 12 ($t(10) = 0.440$, $p = 0.670$).

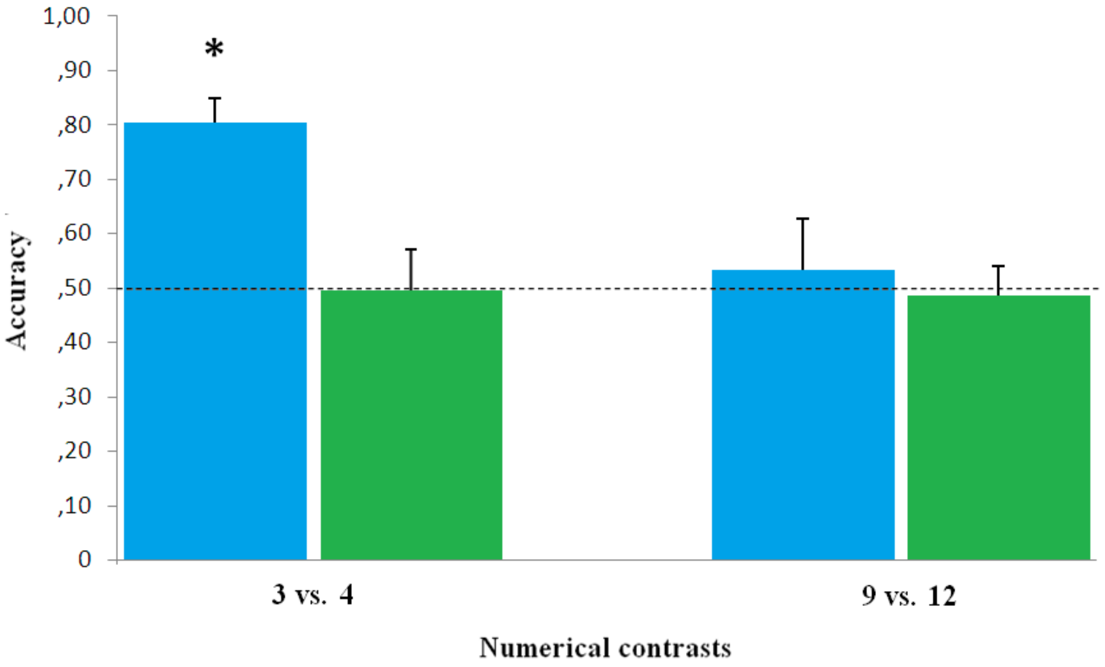


Fig. 30: *Results of the test phase. In the presence of moving stimuli (blue columns), fish successfully discriminated 3 versus 4, but not 9 versus 12. No discrimination for either numerical contrast was found in the presence of static stimuli (green columns). Asterisk denotes a significant departure from chance level (P<0.05). Bars represent the standard error.*

5.2.1. Discussion

Our first finding is that the influence of motion differs as a function of the numerical range examined. Fish trained with the 0.50 ratio showed the same performance for small and large numbers, whether they were tested with static or moving objects. In contrast, when the ratio became more difficult (0.75), items in motion were successfully discriminated in the subitizing range while no effect of motion was found on large-number discrimination.

Different performance for static and moving items has been previously reported in the literature. Humans proved to be faster (Trick et al., 2003) and more accurate (Alston & Humphreys, 2004) at enumerating the number of moving items in the range of 1–4. As concerns comparative psychology, the picture is less clear: it has been shown that old world (rhesus monkeys) and new world (capuchin monkeys) monkeys can discriminate the larger group of items in motion, apparently with the same effort required to discriminate static patterns (Beran, 2008b). However, the performance was not analyzed separately for small and large numbers in that study, making it impossible to assess whether items in motion were better discriminated in the small-number range. Instead, a recent study found that black bears are better able to enumerate static rather than moving stimuli (Vonk & Beran, 2012). The authors referred to the OTS as a potential explanation for their findings: bears are known not to live in social groups and hence they might not have been subject to strong selective pressures in favor of the ability to track individual members of a group. In this sense, their performance would be worse for items in motion. On the contrary, for social species (including guppies), it would be important to track the presence of individual members of a moving group, and the precision of their OTS would be crucial for survival.

A second interesting finding is that, under some conditions, fish can discriminate up to a 0.75 ratio, a performance similar to that reported for most non-human primates (Beran, 2004; Beran et al., 2008b; Cantlon & Brannon, 2007b; Hauser et al., 2000). Previously, discrimination of a 0.75 ratio was reported when fish had to choose between two groups of social companions. However, the latter paradigm did not allow an accurate control for non-numerical continuous variables that co-vary with number; indeed, it was suggested that, in choosing the largest shoal, fish could have used primarily a proxy for number, such as the cumulative area occupied by stimuli (Agrillo et al., 2008a).

For both numerical ranges, the items to be enumerated were identical; the numerical ratio was the same, as well as the way we controlled for the continuous variables. How can we then explain the different effect of the items in motion on the performance for small and large numerosities? Many authors have suggested that the discrimination of a small number of items is generally accomplished by the OTS. This system, shared by human and non-human animals, would primarily serve to keep track of objects present in the perceptual space, (Bisazza et al., 2010; Bonanni et al., 2011; Hauser & Spelke, 2004), but it could be also co-opted to enumerate precisely small quantities of objects producing the subitizing effect. The OTS should respond maximally to moving objects. Indeed it was recently shown that the accuracy of the OTS in keeping track of objects is higher in human and non-human primates for moving items (Matsuno & Tomonaga, 2006). It was suggested that ‘‘motion’’ would be a basic feature in the primate visual system, and that the presence of that basic feature would be more easily detected by the OTS than its absence. Regardless of the exact reason underlying this difference, the better performance of the OTS with respect to items in motion aligns with the hypothesis according to which the activation of the OTS would facilitate better performance in numerical tasks involving small numbers.

While the existence of an ANS in non-human vertebrates is generally accepted, an open debate surrounds the possibility that non-human vertebrates are endowed with a distinct mechanism for enumerating small quantities. To date, evidence supporting the two-system hypothesis comes principally from empirical studies that have compared ratio dependency in small- and large-number discrimination. For instance, Agrillo and colleagues (2012) showed that adult guppies are particularly accurate when required to differentiate the larger shoal from the smaller one in the range of 1–4. Fish successfully discriminated 1 versus 4 (ratio 0.25), 1 versus 3 (0.33), 1 versus 2 (0.50), 2 versus 3 (0.67), and 3 versus 4 fish (0.75) with the same accuracy, thus showing no influence of numerical ratio for small quantities. In contrast, their ability with larger quantities (≥ 4) for the same ratios depended on numerical ratio, with their accuracy decreasing as numerical ratios increased between the small and the larger groups. Similarly, data in line with the two-system hypothesis have been reported for macaques (Flombaum et al., 2005; Hauser et al., 2000), dogs (Bonanni et al., 2011), New Zealand robins (Hunt et al., 2008), and angelfish (Gòmez-Laplaza & Gerlai, 2011 a). Nonetheless, as several authors noted, the different ratio sensitivity for small and large numbers may have alternative explanations. Our results provide further support to the idea of separate numerical systems as a function of numerical range. In addition, the fact that numerical acuity with items in motion is improved only with small numbers suggests the automatic activation of an OTS in fish similar to that described in adult humans.

In our species the automatic engagement of the OTS by items in motion may not be present at birth. Infants fail when comparing sets near the boundary of the OTS range in both moving (Feigenson & Carey, 2005; Feigenson et al., 2002a; van Marle, 2013) and static conditions (Cordes & Brannon, 2009; Xu et al., 2005) and the possibility exists that the automatic activation of the OTS varies across development. This debate extends far beyond the scope of this study. However, future studies on newborn guppies may help us to test this hypothesis, at least in non-human species. It is worth noting that the present method is relatively rapid compared to standard operant conditioning procedures, thus making it possible to study the development of numerical abilities in a rapidly growing species, such as guppies. The influence of motion in the OTS range could now be investigated in newborn and juvenile fish.

As an alternative explanation for the different ratio dependency seen in the two numerical ranges, some authors have suggested that small sets of items usually generate recognizable geometric patterns—the so-called ‘pattern recognition’ hypothesis (Mandler & Shebo, 1982; Neisser, 1967). Our study does not support this view, as we found that items in motion, whose general configuration is continuously dynamic and for which no stable pattern can be easily recognized hence, are better discriminated by comparison to static patterns. One may also argue that better performance with items in motion might reflect the use of a non-numerical cue, namely the total quantity of motion, as a proxy for number (Gòmez-Laplaza & Gerlai, 2012). However, this is not the case, as we did not find any difference in the range of 1–4 (2 vs. 4 and 3 vs. 4) and in 6 versus 12, whether stimuli were controlled or not controlled for the total quantity of motion. In contrast, it is interesting to note that the accuracy in discrimination of 9 versus 12 varies as a function of the type of motion control: when stimuli were controlled for the total quantity of motion, fish performance was significantly lower compared to that observed for the condition in which both number and total motion were congruent and simultaneously available. It is worth noting that several fish species, including guppies, are unable to use numerical information to discriminate a 0.75 ratio in the ANS range (see Section 4.1 and 4.2). In this sense, guppies seem to use the quantity of motion cue when the numerical ratio exceeds their capacity to discriminate by using numerical information only.

Previous evidence has found that in some circumstances human infants are sensitive to continuous quantities in the OTS range (Feigenson et al., 2002a; Wood & Spelke, 2005; Xu, 2003). On the other hand infants repeatedly proved able to discriminate between large quantities by using numerical information only (Lipton & Spelke, 2003; Xu & Spelke, 2000). In our study the performance of guppies was not affected by any of static continuous quantities in both small and large number discrimination. However, as regards the quantity of motion cue, we found a reverse pattern from what would be predicted from the infant data, that is guppies use quantity of motion cue in the ANS range. Despite several similarities have been reported in large number discrimination between the numerical systems of fish and the pre-verbal systems of humans (Agrillo et al., 2010, 2012; Piffer et al., 2012), the possibility remains that the perceptual/cognitive mechanisms involved in the ANS range are at least partially different in the two species.

To summarize, although the present work does not represent direct evidence for the existence of an OTS in fish, it reinforces the idea of separate cognitive systems for small and large numbers. Besides different responses to movement found in the present study and a ratio-insensitivity in the range of 1–4 reported by Agrillo and colleagues (2012), several other lines of evidence support the hypothesis for multiple numerical systems in fish. In spontaneous shoal choice, mosquitofish make use of different continuous quantities for small- and large-number discrimination (Agrillo et al., 2008a); as well, the continuous quantities used by trained mosquitofish to discriminate between sets of two-dimensional figures differed in the two numerical ranges (Agrillo et al., 2009, 2010). In guppies, the developmental trajectory was found to differ for small- and large-number discrimination: the spontaneous ability to discriminate between small quantities of conspecifics is displayed at birth, while this ability appears at 20–40 days old for quantities beyond 4 units (Bisazza et al., 2010). Human infants show an apparent inability to compare quantities across the small- and the large-number boundary, for example, an inability to discriminate 1 from 4 items (the OTS in infants seems to be limited to 3 items, see Feigenson and Carey, 2005). According to some authors, a direct comparison between small numbers (which are supposed to be processed by the OTS) and large numbers (processed by the ANS) would determine a conflict between the two types of representations, leading to poor performance (Cordes & Brannon, 2009). A recent study found that fish may also suffer from the same inability to discriminate across the small-number/large number divide. In shoal choice experiments, guppies were able to discriminate 3 versus 4 fish, but not 3 versus 5 fish, although the latter had a more favorable numerical ratio (Piffer et al., 2012).

Further investigation manipulating other physical features of the stimuli will help us to shed light on this issue. In the meantime, given the absence of alternative theoretical frameworks that might enable us to explain all of the differences reported below and above 4 units, we must admit the possibility that teleost fish, like humans, display the use of different numerical systems for small and large quantities.

6. ONTOGENY OF NUMERICAL ABILITIES

Studying the ontogeny of numerical competence is useful to understand whether a species in naturally endowed with numerical capacities or rather, they emerge over development. The assessment of cognitive abilities often requires that subjects are maintained in social isolation for prolonged periods, raising ethical problems, especially when young individuals are tested. Once set up a proper procedure to study discrimination learning in newborn fish, the development of numerical abilities has been investigated in 4- to 9-old-day guppies.

6.1. Development and application of a new method to investigate cognition in newborn guppies*

** This research is a modified version of "Miletto Petrazzini M. E., Agrillo, C., Piffer, L., Dadda, M., & Bisazza, A. (2012). Behavioural Brain Research, 233, 443-449".*

For a long time, the study of cognitive mechanisms underlying perception, memory, attention, and other higher mental functions has been almost entirely confined to mammals and birds. However, in recent years, it has become evident that even cool-blooded vertebrates and invertebrates can exhibit complex behavior and sophisticated cognitive functions previously believed to be uniquely present in species provided with large, complex brains (reviewed in: Bshary et al., 2002, Brown & Laland, 2003, Bisazza, 2010).

Despite a long-standing tradition of research into the ontogeny of cognitive functions in humans and non-human primates (Matsuzawa, 2007; Haun et al., 2010, Bjorklund et al., 2010), it is only recently that attention has been paid to examine cognitive development in other vertebrate species such as fish (Bisazza et al., 2010), birds (Regolin et al., 1995, Hoffmann et al., 2011), and mammals (Dumas & Doré, 1989, Wills et al., 2010). Fish possess features that make them unique for the study of cognitive development. Unlike mammals and birds, parental care in fish is usually restricted to the prenatal period; therefore, newly hatched fish must carry out many functions that characterize adult life. Hence, newborns are equipped with a behavioral repertoire that enables them to orient in the environment, find food, avoid predators, and interact with conspecifics. However, since newborn fish can be hundreds or thousands of times smaller than adults, there is a considerable ontogenetic niche shift, and the demands imposed by the physical and biological environment early in life could be quite different than those faced in adult life. The small relative size of early stages has a second important consequence. Since brain size in fish steadily increases throughout life (Brandstatter & Kotrschal, 1990, Kihslinger & Nevitt, 2006), at birth, the number of neurons that regulates behavior might be less than one-thousandth that of the adult. While there is evidence that in mammals and birds immature individuals show lower cognitive abilities (Adler & Adler, 1977, Zucca et al., 2007), it is not clear whether these abilities also improve during development in fish. In addition, how young fish solve frequent problems set by the environment—such as avoiding predators, catching prey, or swimming in a coordinated fashion while having extremely different body and brain sizes—remains a largely unexplored area of investigation (Kotrschal et al., 1990).

From an experimental point of view, working with newborns that lead an independent life allows for easy manipulation of environmental factors that might affect cognitive development, such as experience. This experimental intervention is extremely problematic in species such as mammals and birds that are characterized by a prolonged association with the parents (Bisazza et al., 2010, Freire et al., 2004).

Training procedures represent one of the main tools for studying cognitive capacities in animals. Existing training paradigms normally require that subjects be maintained in social isolation for prolonged periods; however, isolation may represent an unnatural condition for social species, especially when research focuses on newborns and young individuals. Social isolation could be a source of distress, interfering with cognitive functioning and negatively affecting the normal development of the nervous system (Frisone et al., 2002; Lu et al., 2003; Tsoory et al, 2007).

Most teleost species are social and live in aggregates. There are several possible advantages associated with living in groups. Individuals in shoals can be safer than solitary fish (Hager & Helfman, 1991), have more opportunity to access mates (Agrillo et al., 2008b), or enjoy hydrodynamic advantages during locomotion (Barber & Folstad, 2000). As group size increases, however, some disadvantages might arise, such as an increased competition for food or increased risk of disease transmission. Due to this trade-off, shoaling behavior is often context dependent. Hoare et al. (2004), for example, found that banded killifish (*Fundulus diaphanus*) formed larger groups in the presence of predators but increased inter-individual distance when competing for food.

Little is known about social behavior in early stages of development in fish. In some species, newborns appear to be solitary and their social behavior tends to increase across development. In silversides (*Menidia menidia*), schooling behavior starts when they are about twenty days old and develops gradually (Shaw, 1960, Shaw, 1961). In anchovy larvae (*Engraulis mordax*), schooling becomes clearly established when they are about thirty days old (Hunter & Coyne, 1982). Recently, Buske and Gerlai (2011) performed a laboratory study of the development of shoaling behavior in zebrafish (*Danio rerio*) from day seven post-fertilization to over five months of age, reporting that a few days after hatching fish do not form cohesive shoals, but shoaling tendency increases during development.

Observations made in a laboratory suggest that unmanipulated newborn guppies show little tendency to aggregate (Bisazza, personal observation). On the other hand, several studies reported that when newborn guppies are placed in an unfamiliar environment or are exposed to predation risk, they gather and form schools (Bisazza et al., 2010; Evans et al., 2004) a tendency that appears to be greater for guppies from high-predation sites (Magurran & Seghers, 1990). Therefore, while it seems established that young guppies become gregarious in potentially hazardous situations, it is not clear how they behave when the environment poses no immediate threat.

The present study aimed to investigate early social development in guppies and design new learning procedures for young fish that minimize stress from social deprivation. To achieve our goal, in the first part of the study (Experiments 1 and 2), we analyzed the development of social behavior in newborn guppies. Experiment 1 was designed to measure the gregarious tendency of guppies from birth for two weeks in a three-compartment choice apparatus in which subjects could choose between a compartment containing three stimulus fish and an identical but empty compartment. Experiment 2 investigated the preference for either real conspecifics or their own mirror image in guppies from birth to sixteen days of age to assess the possibility of using mirrors as a substitute for social companions. Based on the findings of these experiments, in the second part of the work, we designed a procedure for studying learning which took into account the social needs of young fish (Experiments 3–4). Experiment 3 tested whether young fish could be trained to associate a stimulus with a food reward. Experiment 4 was set up to test whether young fish can discriminate between two different geometric figures to get a food reward.

6.1.1. Experiment 1: Ontogeny of gregarious behavior

In the first experiment, we measured the gregarious tendency of guppies in a familiar environment from birth for two weeks in a three-compartment choice apparatus in which they could choose between a compartment containing three stimulus fish and an identical but empty compartment.

Subjects

The fish used in this experiment were descendants of wild-caught fish from the Lower Tacarigua River (Trinidad), a high-predation location. A total of six newborn guppies were tested and eighteen newborn guppies were used as stimuli. As reference, we used a sample of eight adult females (twenty-four social stimuli). Female guppies are highly social and commonly form shoals of variable sizes in their natural environment.

To collect newborn individuals, we placed females close to parturition alone in nursery tanks (50 cm x 18 cm x 32 cm). After parturition, newborns were transferred to the apparatus for the habituation phase.

Apparatus

The apparatus consisted of a tank (50 cm x 18 cm x 32 cm) divided into three compartments by two transparent plastic partitions (Fig. 31). The central one, the "subject compartment" (28 cm x 18 cm x 32 cm) housed the test fish. At the two ends, two smaller "stimulus compartments" (11 cm x 18 cm x 32 cm) faced the subject compartment. Two small holes (1 cm Ø) in the lower part of each transparent plastic partition allowed water to flow between compartments. There was a plant as shelter for the test fish in the center of the subject compartment. Two green tiles (16 cm x 10 cm), placed adjacent to the stimulus compartment, delimited the "preference area". The walls of the tank were externally covered with green plastic to prevent stimulus fish and subjects from seeing outside. The bottom of the tank was covered with natural gravel, and the water was maintained at a temperature of 25 ± 2 °C. Each stimulus compartment was lit by a 15 W fluorescent light, and the subject compartment received light indirectly from them. We placed the apparatus in a darkened room, and placed a transparent glass above the subject compartment to prevent the fish from seeing outside. We suspended a video camera about one meter above the apparatus and used it to record the position of the subjects during the test. The apparatus for adult females was similar

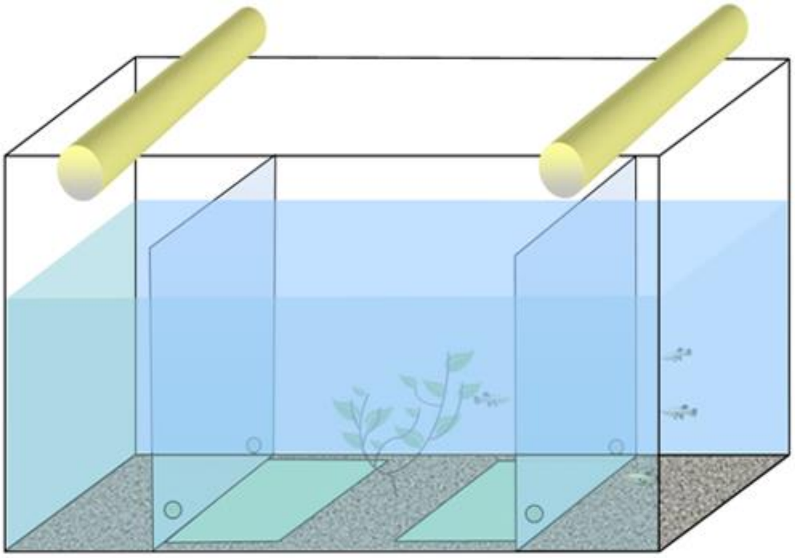


Fig. 31: *Schematic representation of the experimental apparatus. To measure their gregariousness, newborn guppies were singly inserted in the middle of the tank and observed in a free choice between a compartment containing three conspecifics and an identical but empty one.*

to the one used for newborns, but larger in size (90 cm x 40 cm x 34 cm) and with larger preference areas (19 cm x 15.5 cm).

Procedure

At birth, one newborn guppy was introduced in the subject compartment and three conspecifics were introduced in one of the two lateral stimulus compartments. The other compartment remained empty. In half of the trials, the stimuli were on the left and in the other half, they were on the right. Observations started the next morning and were repeated for fifteen consecutive days. Each day, the subject was video recorded for a total of 6 h, subdivided into three sessions. Each session consisted of two periods of 60 min each, one preceding feeding (pre-feeding phase) and one (post-feeding phase) 20 min after each of three daily meals, consisting of a drop of live brine shrimp (*Artemia salina*). There was a 1-h interval between each session. The same procedure was used with adult females; the only difference was that they were introduced in the apparatus 24 h before the test and were tested only for one day.

From the video recordings, we scored the time spent by the subject in the preference areas near the compartment containing social stimuli and near the empty compartment. The observer of this video was blind with respect to the aim of the experiment. As measure of gregariousness, we computed the proportion of time spent near social stimuli in the preference area. As the data from this and subsequent experiments did not significantly deviate from normality (Kolmogorov–Smirnov test, $p > 0.05$) we used parametric statistics. Statistical tests were carried out using SPSS 18.0.

Results

Adult females spent significantly more time near conspecifics than near the empty compartment (mean ± SD: 0.715 ± 0.097; one-sample *t*-test, $t(7) = 6.25$, $p < 0.001$; Fig. 32). Young fish also spent more time near conspecifics (mean ± SD: 0.60 ± 0.063; one sample *t*-test, $t(5) = 3.87$, $p = 0.012$), although they were, on average, less gregarious than adults (independent *t*-test, $t(12) = 2.51$, $p = 0.027$). The two conditions were not fully comparable, since adults were tested after 24 h of acclimation to the apparatus, while young fish stayed for fifteen days. However, the same difference was found comparing adults with one-day olds (i.e. fish with the same acclimation time) (independent *t*-test, $t(12) = 4.91$, $p < 0.001$). A 2 x 15 repeated measure ANOVA with feeding condition (pre-feeding and post-feeding phase) and age (from one- to sixteen-day-old) as within-subject factors showed that the gregariousness of young guppies varied during the experiment and they were significantly more gregarious after meal (feeding condition: $F(1,5) = 8.03$, $p = 0.037$; age: $F(14,70) = 1.96$, $p = 0.034$, interaction: $F(14,70) = 0.80$, $p = 0.663$; Fig. 33). No difference in gregarious tendency was found in adult guppies before and after meals (paired *t*-test, $t(7) = 1.66$, $p = 0.141$). The results were not affected by the left or right position of the stimuli (independent *t*-test, $t(4) = 1.59$, $p = 0.186$).

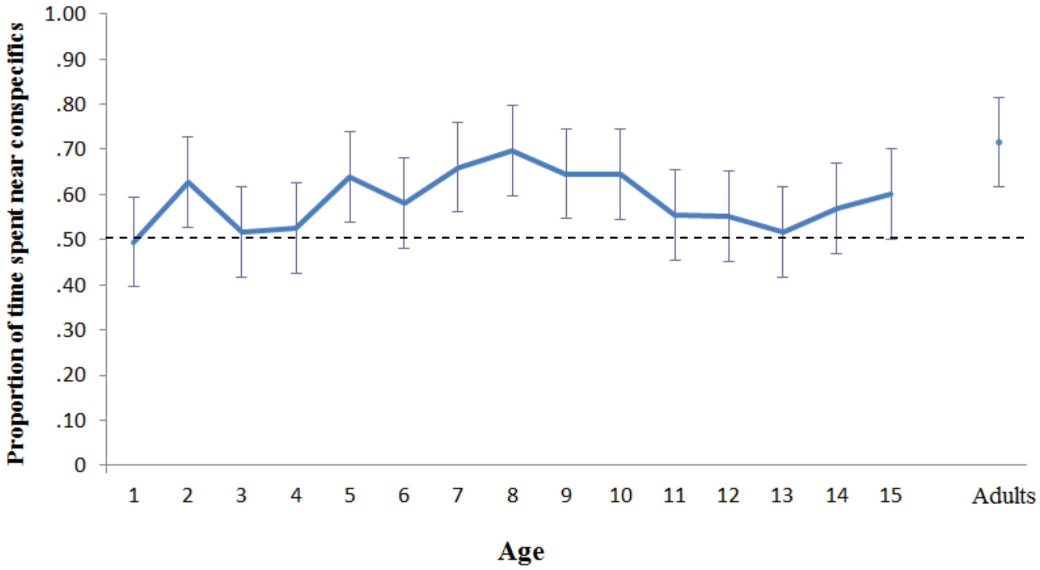


Figure 32: *Preference for conspecifics vs. an empty compartment in one- to fifteen-day-old fish and in adults. On the whole, young guppies were significantly less gregarious than adult fish.*

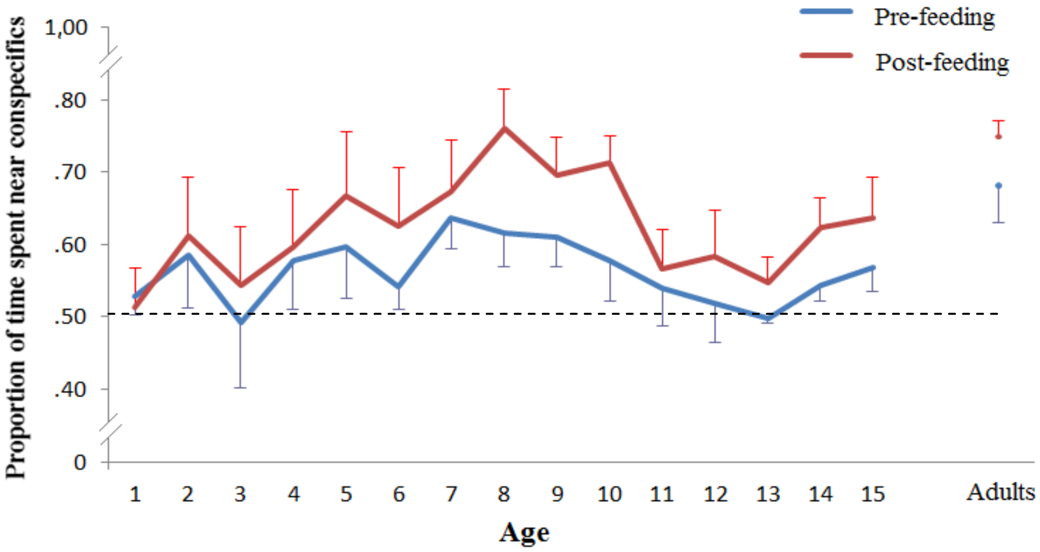


Fig. 33: *Preference for conspecifics vs. an empty compartment before and after a meal. Satiation tended to increase gregariousness. Bars in this and in the following graphs represent the standard error.*

6.1.2. Experiment 2: Preference between mirror image and real conspecifics

Previous studies have shown that mirror images can be used as a substitute for conspecific in adult fish in different teleost species (Milinski, 1988; Meliska et al., 1980; Dugatkin & Alfieri, 1991; Sovrano et al., 2001; Bisazza & De Santi, 2003; Dadda et al., 2010). No data are available for young fish. In the second experiment, we investigated the preference for either real conspecifics or their own mirror image in guppies from birth to sixteen days of age to assess the possibility of using mirrors as a substitute for social companions for newborns and young individuals.

Subjects and apparatus

A total of fifty fish were used as subjects of this experiment. Fish were tested at one, four, eight, and sixteen days of age and as adults (five- to eight-month-old guppies). Each age group consisted of ten fish. One hundred additional fish were used as stimuli. The apparatus was similar to that used in Experiment 1; it differed from the previous apparatus since there was no plant in the middle subject compartment, and the tank was lit by a single 15 W fluorescent light above the subject compartment. Furthermore, one of the two stimulus compartments was substituted by a mirror so the fish could choose between one compartment with two conspecifics and one with its own mirror image. In addition, we placed two small mirrors (29 cm x 5 cm) at the sides of the main mirror to partly compensate for the fact that the subject chose between two real fish and a single virtual one (Fig. 34). In this way, when the subject was near the corner, it saw two virtual fish. Adult females were tested in an apparatus similar to the one used for new-borns but larger in size (90 cm x 40 cm x 34 cm) and with larger preference areas (19 cm x 15.5 cm). We placed the tanks in a dark room to eliminate extra-tank cues.

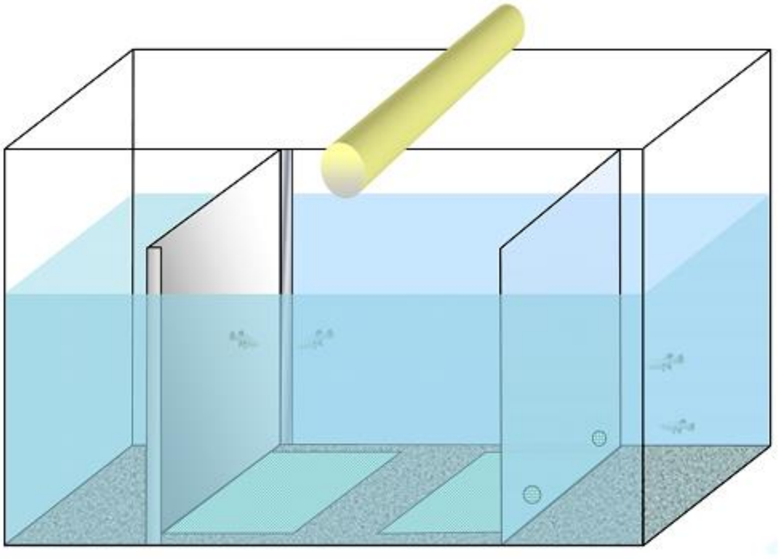


Fig. 34: *The apparatus was the same used as in Experiment 1, but the empty compartment was replaced by a mirror to test whether mirrors can be used as a substitute for conspecifics.*

Procedure

Two fish were introduced into the stimulus compartment to habituate for 1 h. A subject was then introduced by a green net into the subject compartment so that the fish was approximately halfway between the stimulus fish and the apparent mirror image position. It was allowed to familiarize for 10 min, and then its behavior was recorded for 50 min. From video recordings, we calculated social tendency as the proportion of time spent near the two social stimuli (conspecifics or mirror image); whereas, as measure of preference, we computed the proportion of time spent near real conspecifics.

Results

Overall, the subjects of all age groups spent more than 80% of their time near one of the two social stimuli (average; mean ± SD: 0.892 ± 0.088). No significant difference in social tendency was found among age groups (ANOVA *F*(4,49) = 2.04, *p* = 0.105). No preference for either the mirror image or real conspecifics was observed in adults (one sample *t*-test, *t*(9) = 0.93, *p* = 0.375) and young fish, either when considering the young group as a whole (one sample t-test, *t*(39) = 1.95, *p* = 0.058) or taking into account each age group separately (one-sample *t*-test; one day old: *t*(9) = 0.98, *p* = 0.351; four days old: *t*(9) = 1.78, *p* = 0.108; eight days old: *t*(9) = 1.01, *p* = 0.338; six-teen days old: *t*(9) = 0.11, *p* = 0.915; Fig. 35). We found no side bias (left vs. right end of the tank, independent *t*-test, *t*(38) = 1.28, *p* = 0.210). There was no difference among groups in the preference for the mirror image (ANOVA *F*(4,49) = 0.26, *p* = 0.901). Analysis of trend also evidenced no age-related variation in preference (trend analysis *F*(1,49) = 0.06, *p* = 0.8

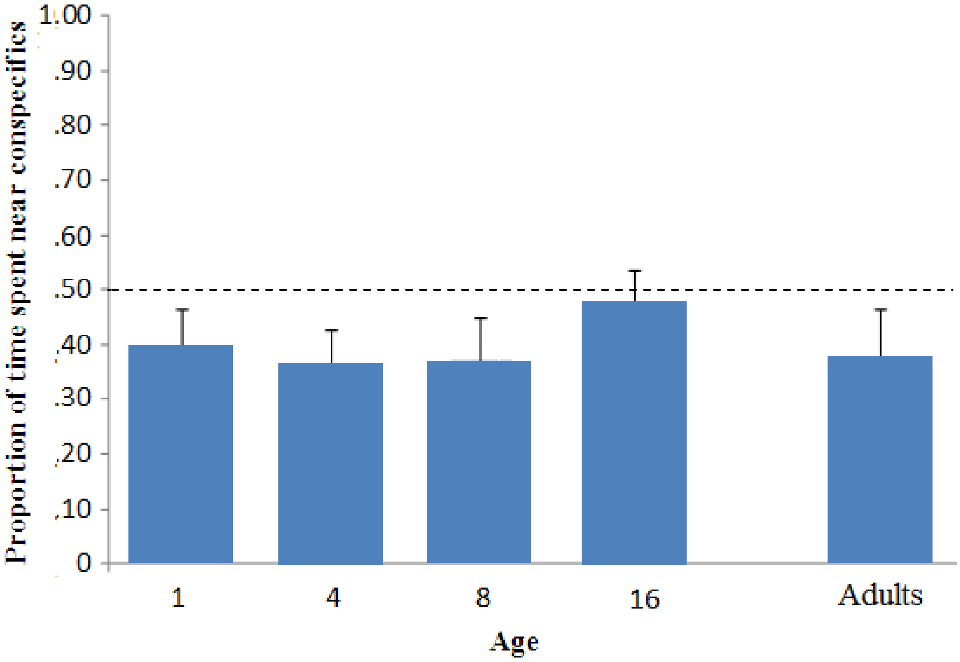


Fig. 35 : *Results of Experiment 2. Preference for real conspecifics vs. mirror image at different ages. No significant difference was found among the five age groups and no group showed a significant preference for one stimulus type.*

6.1.3. Experiment 3: Learning the association between a stimulus and food reinforcement

The aim of this experiment was to determine if young fish could be trained to associate a stimulus to a food reward. We adapted the procedure set up for adult fish (see Section 4.1) Subjects were repeatedly fed in the proximity of a stimulus placed at one of the two ends of the tank. As measure of the capacity to associate the stimulus with food reinforcement, we computed the proportion of time spent near the stimulus during probe trials in which no reward was provided. To reduce stress from social deprivation to a minimum, we accustomed fish to the apparatus and initially trained them in a group. In addition, we placed two mirrors in the middle of the tank as a substitute for social mates.

Subjects and apparatus

We used twelve four-day-old fish in this experiment. The apparatus consisted of a tank (28 cm x 18.5 cm x 32 cm) filled with 17 cm of water and lit by a 15 W fluorescent light (Fig. 36). Two green plastic panels on each short side of the tank delimitated the area where the stimulus was introduced. Two green tiles (16 cm x 10 cm) placed adjacent to each short side delimited the “preference area”. The bottom of the tank was covered with natural gravel, and the water was maintained at a temperature of 25 ± 2 ◦C. The stimulus was a black cross (3 cm x 3 cm) on a white background (6 cm x 29 cm). We used two Pasteur pipettes to provide both a drop of live brine shrimp (*Artemia salina*) and a drop of water at the two ends of the apparatus. We placed the tank in a dark room. We video recorded the experiment.

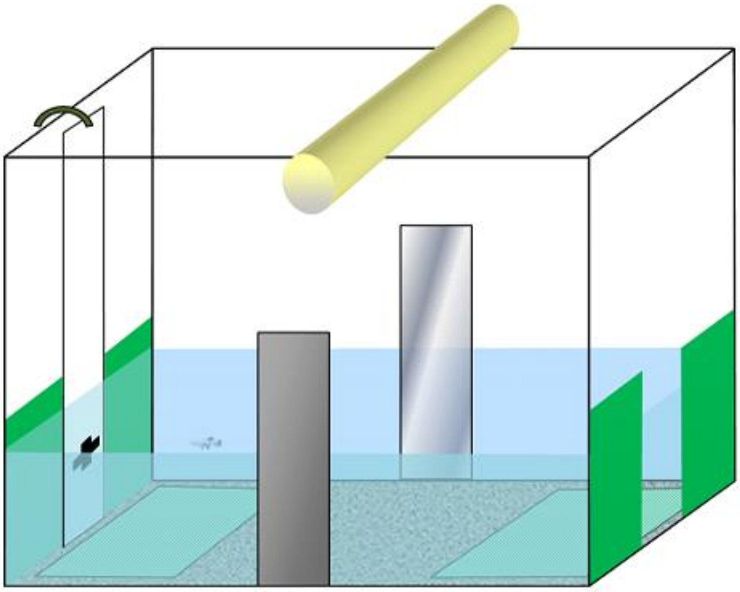


Fig. 36: *Schematic representation of the experimental apparatus. In Experiments 3 and 4 subjects were required to learn an association between a stimulus (a two-dimensional geometric figure) and a food reward (Experiment 3), or learn to discriminate between two geometric figures placed at the two ends of their apparatus (Experiment 4).*

Procedure

We introduced four fish in a group into the apparatus the day before the test and allowed them to familiarize for 15 h. The training phase began when fish were four days old. It consisted of four daily reinforced trials for four consecutive days, for a total of sixteen trials. On days 1–2, fish were tested in a group (four individuals). On the evening of day 2, each fish was placed individually in an apparatus identical to the one used for training in a group. On days 3–4, fish were tested singly. To avoid the possibility of fish using the local/spatial cues of their tank, at the end of each day, each subject was moved from one tank to another. This also served to expose subjects to the social odour of the fish previously occupying the tank.

At the beginning of each reinforced trial, we introduced the stimulus at one of the two ends of the tank. Two Pasteur pipettes were simultaneously introduced: one released a drop of *A. salina* near the stimulus, the other a drop of water at the opposite side. After 6 min, a second reinforcement was given. Each trial lasted 12 min and the inter-trial interval lasted 3 h.

On day 5, fish were subjected to three probe trials, alternated with two rein-forced trials to avoid a decrease in their motivation. There was a 1-h interval between the probe trial and the reinforced trial and then a 3-h interval before the successive probe trial. In the probe trial, the stimulus was introduced at one end of the tank with no reinforcement provided. The probe trial lasted 6 min and was video recorded. The position of the stimulus was counterbalanced over trials, both in reinforced and in probe trials. As measure of preference, we calculated the proportion of time spent near the stimulus in the preference area during probe trials.

Results

Fish spent significantly more time near the stimulus in each probe trial (one sample t-test, respectively: $t(10) = 3.93$, $p = 0.003$; t(10) = 3.44, $p = 0.006$; $t(11) = 4.29$, $p = 0.001$). On the whole, the preference for the side with the stimulus was significant (mean ± SD: 0.660 ± 0.101, one sample *t*-test, $t(11) = 5.48$, $p < 0.001$, Fig. 38). We found no side bias (left vs. right end of the tank) (paired *t*-test, $t(10) = 0.20$, $p = 0.846$).

6.1.4. Experiment 4: Learning to discriminate between two stimuli

This experiment aimed to verify whether young fish could be trained to discriminate two stimuli. The procedure was similar to the one used in Experiment 3, but here two stimuli were simultaneously inserted at the two ends of the tank, and only one was reinforced. To study learning in the shortest possible time interval, training was preceded by a two-day pre-treatment phase in which fish learned in a group to receive food near an introduced stimulus.

Subjects and apparatus

A sample of ten four-day-old fish was used in this experiment. The apparatus was the same as in Experiment 4 (Fig. 37). The two stimuli to discriminate were a circle (3 cm x 3 cm) and a triangle (3 cm x 3 cm) of the same colour (black) and on the same white background (6 cm x 29 cm). For the pre-treatment phase we used a black cross, the same stimulus used in Experiment 3.

Procedure

In a pre-treatment phase lasting two days, we used an identical procedure to the one used in the previous experiment. We trained fish in a group of five individuals to receive food in the proximity of a stimulus (cross). At the end of day 2, we placed the fish individually in an apparatus equal to the one used for training in group. On days 3–5, fish were trained singly to discriminate between two new stimuli (circle and triangle) for four daily trials for three consecutive days (for a total of twelve trials). On day 6, fish were subjected to three probe trials alternated with two reinforced trials to avoid a decrease in their motivation. In probe trials, we introduced stimuli at the two ends of the tank for 6 minutes and provided no reinforcement. The position of the stimuli was counterbalanced over the trials, both in reinforced and in probe trials. Half of the fish were trained with the circle, and the other half were trained with the triangle as positive.

As measure of preference, we calculated the proportion of time spent near the reinforced stimulus in the preference area.

Results

Fish spent significantly more time near the reinforced stimulus in the third probe trial (mean ± SD: 0.797 ± 0.142, one sample *t*-test, *t*(8) = 6.25, $p < 0.001$) but not in the other two ($p > 0.05$). A repeated measure ANOVA with Trial (1st/2nd/3rd probe trial) as within factor showed that fish performance increased in precision across trials (*F*(2,16) = 8.36, $p = 0.003$; linear trend, *F*(1,8) = 7.62, $p = 0.025$). On the whole, the preference for the side with the stimulus was significant (mean ± SD: 0.624 ± 0.076, one sample *t*-test, *t*(9) = 5.10, $p = 0.001$, Fig. 37).

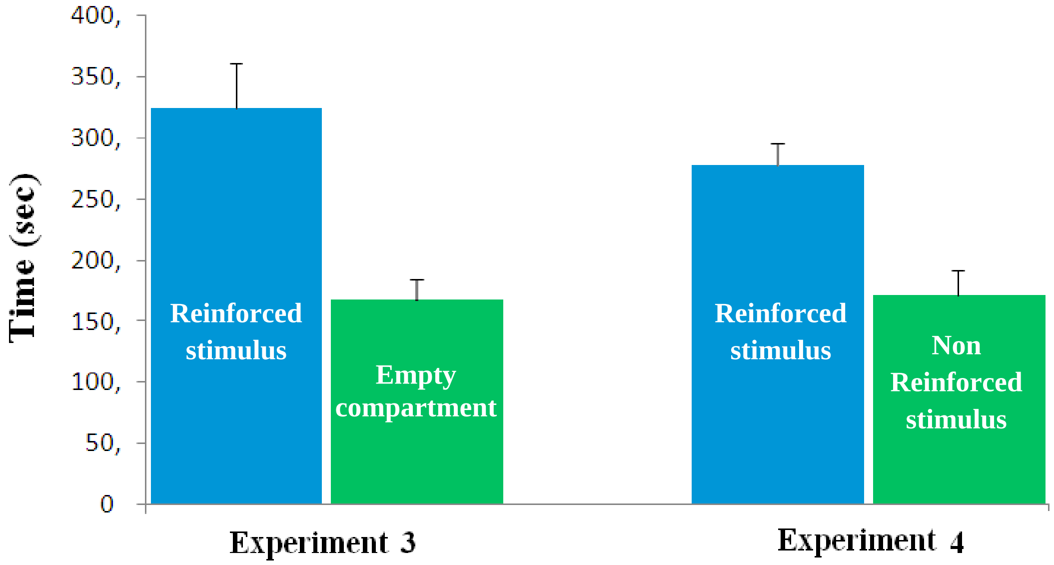


Fig. 37: *Results of Experiments 3 and 4. Time spent near the reinforced stimulus in probe trials was used to demonstrate the ability of young guppies to learn the association between one stimulus and food reward (Experiment 3), and the discrimination between two stimuli (a circle and a triangle) (Experiment 4).*

6.1.5. Discussion

In the first two weeks of their life, guppies spent significantly more time near a compartment containing a group of same-age conspecifics than near an empty compartment. However, the preference was small, and young guppies were found to be significantly less gregarious than adults tested in a similar condition. One could argue that this difference in gregariousness may derive from the fact that adults were essayed after 24 h of acclimation, while young stayed in the apparatus for two weeks. Yet the significant results found comparing adults with young fish in their first day of experiment (i.e. having had the same acclimation time as adults) indicated the diversity was real. Interestingly, a recent investigation on the ontogeny of shoaling behavior in zebrafish reported that these fish also showed very little tendency to shoal in their first week of life and that shoal cohesion progressively increased in successive weeks, reaching its maximum value in adulthood (Buske & Gerlai, 2011).

Although group living provides several benefits, competition for food increases with increasing shoal size (Bertram, 1978). As a consequence, fish increase inter-individual distances (Morgan, 1988; Robinson & Pitcher, 1989) or prefer foraging alone or in small groups when hungry (Hoare et al., 2004). A similar effect was observed in our experiment: when we compared pre-feeding and post-feeding observations, we found that juvenile guppies increased their proximity to conspecifics after they had consumed a meal. Interestingly, this behavior was not observed in adults. It might be that competition for food is higher in young individuals due to their need to allocate considerable resources for somatic growth. However, it is also possible that a prolonged experience of “ad libitum” feeding in the laboratory had led adults to reduce their response to food competition.

The reasons underlying the change of gregariousness between early life and adulthood are not clear. It is possible that adult and newborn fish are subjected to differential selection pressure. Some predators prefer to attack a single prey when it is large, but they attempt to catch a group of individuals when they are small (Anderson, 2010; Holmes & McCormick, 2009). In such cases, forming a cohesive group might be more advantageous for adult fish than for newborns. There is no information about the importance of predation on young guppies in our population. The Lower Tacarigua is a high-predation site where guppies coexist with large predators, including the pike cichlid, *Crenicichla alta* (Magurran & Seghers, 1994). However, predation risk of the newborns does not necessarily reflect that of adult fish. Indeed, in poeciliids, cannibalism often represents the major cause of mortality of larval fish (Loeckle, 1982; Nilsson et al., 2011); hence, high predation pressure on adults, by reducing population densities and competition for resources, might actually release immature fish from intraspecific predation.

Social tendency was not uniform throughout the first two weeks of life. In particular, there appeared to be less tendency to aggregate with other fish in the first days of life, but due to our experimental design it was impossible to determine whether this difference was due to development or to habituation to the experimental condition. Despite some sociability differences related to age and satiation levels, it was evident from our experiment that newly born guppies did not exhibit a clear solitary tendency even when they were observed undisturbed in a familiar environment. Based on these results we cannot exclude that isolation of young guppies for a prolonged period might represent a stressful condition, negatively affect learning, or even interfere with normal development of their behavior. Further experiments (e.g. measuring cortisol levels, assessing behavioral development, etc.) are needed to unravel this point.

Mirror images have often been employed to simulate conspecific fish in studies on cooperation (Milinski, 1988; Dugatkin & Alfieri, 1991), aggression (Meliska, 1980; Bisazza & De Santi, 2003), and schooling (Sovrano et al., 2001; Dadda et al., 2010). These studies have generally assumed that no difference occurred between virtual and real fish. A notable exception was a recent work in African cichlid fish (Desjardins & Fernald, 2010) reporting that, in spite of no evident behavioral difference, distinct brain gene expression levels were observed when fish fight a mirror image or a real conspecific. In Experiment 2, when given the choice between conspecifics and their own mirror images, young guppies and adult females showed no preference for real fish over virtual ones. If anything, there was a slight preference for the mirror side that appeared more evident in younger fish. This finding opens the possibility of using mirrors as a substitute for social companions to avoid social deprivation during experiments that require individual housing of the subject.

In the second part of this study, we designed a procedure to study discrimination learning in newborn and young fish, taking into account the results of Experiments 1 and 2 to develop protocols to minimize potential stress deriving from social isolation. We reduced to a minimum the time spent in isolation by keeping the subjects in a group during the period of habituation to the new apparatus. We placed two mirrors in each tank to provide social stimulation. There is now increasing evidence that conspecific odour is an important social cue in guppies (Brown & Godin, 1999, Griffiths & Magurran, 1999; Shohet & Watt, 2004). Consequently, we decided to provide some olfactory stimulation during all the trials.

The aim of Experiments 3 and 4 was to determine whether fish could be trained at early stage of development. In Experiment 3, we trained fish to associate a stimulus with a food reward and found that eight-day-old fish can learn to go to the side of the apparatus where the stimulus appears after only sixteen reinforced trials. In Experiment 4, we modified this procedure to determine if fish could discriminate between two geometric figures, a triangle and a circle. In this task, nine-day-old guppies showed a significant selection of the reinforced stimulus after only twelve reinforced trials. Shape discrimination has been previously studied in adult fish (Hemmings & Matthew, 1963; Hemmings, 1965: Sutherland, 1969; Sovrano & Bisazza, 2008; Siebeck et al., 2009) but no data are available in young individuals. Here, we provide the first evidence of geometric figure discrimination in newborn guppies using a training procedure. The demonstration that very young fish can discriminate two geometric figures after only a few reinforced trials paves the way for future studies on inborn cognitive abilities of fish as well as the possibility to study the roles that experience, maturation, and increase in relative brain size have on their development. In particular, it would be interesting to investigate whether young fish exhibit the same cognitive abilities of adults with regard to other visual capacities such as colour discrimination (Muntz & Cronly, 1966; Colwill et al., 2005), shape discrimination (Siebeck et al., 2009, Mackintosh & Sutherland, 1963), illusory contour perception (Sovrano & Bisazza, 2009), and numerical abilities (Agrillo et al., 2009; Agrillo et al., 2010).

6.2. Ontogeny of the capacity to compare discrete quantities in fish*

** This research is a modified version of "Miletto Petrazzini, M. E., Agrillo, C., Piffer, L., & Bisazza, A. (2013). Online first"*

Developmental and comparative studies suggest that our complex mathematical capabilities are rooted in foundational non-symbolic numerical systems that are present well before the acquisition of language (Izard et al., 2009; Xu & Spelke, 2000) and that we share with non-human primates (Cantlon & Brannon, 2007b; Nieder, 2013).

Over the last few decades, basic numerical abilities have been demonstrated for several other vertebrates but results across studies are not entirely consistent. It is unclear whether these differences are related to the species investigated or rather they are due to the context in which they are investigated, the type of task presented or the sensory modality involved. In particular, since very few studies have examined numerical abilities in the same species across different contexts, it is not clear whether animals are equipped with a single system of numerical representation, or rather possess domain-specific numerical systems with distinct characteristics. Some studies support the existence of a single supramodal and domain-independent numerical system in newborns and infants (Izard et al., 2009; Starkey et al., 1990) and in non-human primates (Jordan et al., 2005). Other authors however have suggested the existence of multiple core number systems which serve to solve a limited set of problems (Feigenson et al., 2004; Spelke, 2000).

Exploring the development of numerical abilities in different contexts may provide important insights into the nature of these capacities and help us to assess whether the same or distinct numerical systems are used in the different tasks. If a species is endowed with a single system of numerical representation, one would expect to observe the same developmental trajectories for all numerical tasks. Conversely, a difference in the onset of numerical abilities would suggest the existence of domain-specific systems for numerical processing.

In laboratory, fish placed in a novel place readily choose the more numerous of two social groups (mosquitofish: Agrillo, et al., 2008a; guppies: Agrillo, et al., 2012; zebrafish: Pritchard et al., 2001; angelfish: Gómez-Laplaza & Gerlai, 2011a, b). Fish are very efficient at this task, showing numerical abilities comparable or even superior to those of many mammals and birds. When confronted with small numerosities, mosquitofish can discriminate 3 from 4 fish. When the number of fish to discriminate exceeds four, they require a numerical ratio of approximately 1:2, but apparently with no set size effect, at least up to 8 versus 16 fish (Agrillo et al., 2008a). Although they spontaneously make use of continuous quantities that co-vary with number, they are able to choose the larger group even when they see one fish at time, a condition that is assumed to prevent the estimate of these continuous variables (Dadda et al., 2009).

Good numerical abilities were found also by training fish to discriminate between two-dimensional patterns of geometric figures with stimuli controlled for non-numerical continuous quantities (Agrillo et al., 2009, 2010). When numbers differ by one unit, fish discriminate up to 2 versus 3 items (see Section 4.1). For larger numbers in all studied species (mosquitofish, guppies, redtail splitfin, zebrafish, and Siamese fighting fish) the upper ratio appears to be 2:3, for example, 8 versus 12 dots (see Section 4.1 and 4.2); there seems to be no total set size limit, since discriminating 100 from 200 items seems equally easy than discriminating eight from sixteen items (see Section 4.1). Some differences emerged between results obtained with spontaneous shoal choice tests and training procedure. Fish for example are more precise in shoal choice with small numbers and with discrimination learning in large numerosities. It is not clear at the present whether these differences are due to one or more factors that differentiate the two tasks, to the nature of stimuli (live 3D moving stimuli vs. 2D static figures) or rather they reflect the existence of distinct numerical systems operating in two contexts.

A recent study investigated the development of the ability to discriminate number of peers from birth to adulthood in the guppy, a popular model species in behavioral research. One-day-old guppies proved able to discriminate between shoals when the choice involved small quantities (1-4) not when larger groups were presented (4 vs. 5, 4 vs. 8 or even 4 vs. 12). Such abilities seem to emerge only later, between 20 and 40 days of age, as result of maturation and social experience (Bisazza, et al., 2010). As in adults, the capacity to select the larger group was preserved when newborn and young fish were tested under a condition that strongly reduced their possibility to use non-numerical continuous quantities, such as total area or density of conspecifics, suggesting that the capacity to discriminate small quantities of conspecifics at birth might be based on a true numerical process (Bisazza et al., 2010). In the previous study it was shown that guppies can be trained, starting from the fourth day of age, to discriminate between two stimuli in only 12 reinforced trials (see Section 6.1). The method diverges from a standard operant conditioning procedure; in this method discrimination is inferred from the fraction of time spent near the trained stimulus during final probe trials instead of from individual learning criterion. However the procedure is very rapid and allows to study age-specific cognitive abilities even in a rapidly growing species.

Here we applied this method to investigate the numerical abilities of young guppies. In the first experiment, 4-day-old guppies were trained to discriminate between two groups of two-dimensional geometric figures (1 vs. 4, 2 vs. 4, and 2 vs. 3 black dots) under a condition in which they could use both numbers and continuous quantities that co-varied with numerosity (e.g., area). In a second experiment they were trained on the same quantities when only numerical information was available. In experiment 3 they had to discriminate between stimuli that differed in area but not in number.

6.2.1. Experiment 1: Number and continuous quantities

This experiment investigated whether young guppies can learn to discriminate between two sets of dots in the range 1–4. Stimuli were not controlled for the continuous quantities that co-vary with number and thus fish of this experiment could use both number and continuous quantities to solve the task.

Subjects, apparatus and stimuli

Thirty 4-day-old fish were used as subjects. Gravid females were singly placed in nursery tanks (50 cm x 18 cm x 32 cm). The fry were removed at birth and kept in groups until the experiment.

Apparatus and procedure were the same as described in Section 6.1.3. Each subject was tested in a 28 cm x 18 x 32 cm tank filled with 17 cm of water maintained at a temperature of 25 ± 2°C and lit by a 15 W fluorescent light. To minimize the potential stress from social isolation two mirrors (6 cm x 29 cm) were placed in front of each other in the middle of the tank. The tanks were placed in a dark room. A video camera was suspended about 1 m above the experimental tanks and used to record the position of the subjects during the tests.

Stimuli were black dots (diameter 0.29–0.61 cm) on a white background (6 cm x 29 cm) distributed in a 5.5 cm x 5.5 cm central area. There were three ratio conditions (n = 10 each), presenting contrasts of 1 versus 4, 2 versus 4, and 2 versus 3 (respectively, 0.25, 0.50, and 0.67 ratios). Dots have different sizes within each array; however, as stimuli were not controlled for continuous quantities, in 2 versus 3 condition, for example, the total area occupied by the smaller stimulus was 2/3 of the larger stimulus and could cue the larger quantity.

Procedure

Five 3-day-old fish were placed together into the apparatus 15 hours before the beginning of the training in order to familiarize them with the novel environment.

The fish were trained for 5 consecutive days (from the 4th to 8th days of age), in four trials per day. On days 1–2, the fish were trained in groups of five; at the end of day 2, each fish was singly housed and individually tested for the remaining 3 days. To avoid the use of local cues in the tank, at the end of each day, subjects were gently moved by a green net from one tank to another. Fish were moved only in the late evening, after the daily session and trained or tested the following morning (at least 13 hours after being transferred from one tank to another). In this way the potential stress due to the daily transfer was minimized during the experiments as a previous study (see Section 4.1) showed that the daily transfer does not affect discriminative learning.

In each trial, the two stimuli for discrimination were introduced at the two ends of the apparatus immediately followed by the release of a drop of water containing live brine shrimps near the reinforced stimulus and a control water drop near the other. A second food reinforcement was given after 6 min. Each trial lasted 12 min with a 3-hour inter-trial interval. The left/right position of the stimuli was counterbalanced over trials. All subjects were reinforced with respect to the larger quantity and tested only once. On day 6 (9 days of age) subjects received a probe trial followed by three reinforced trials. A second probe trial was administered on day 7, swapping the position of the stimuli. During the probe trials, stimuli were introduced into the tank but no food was provided.

Only drops of waters were inserted in both sides. Probe trials lasted 6 min and were video-recorded. As dependent variable, we computed the proportion of time (accuracy) spent in the preference area (white rectangles covered by a green net, 16 cm x 10 cm) adjacent to the larger stimulus in the average of the two probe trials.

One-way analyses of variance (ANOVA) were performed to compare potential proportion differences among the three ratios; for each ratio, one sample *t*-tests were used to compare the proportion of time in the preference area against a chance value of .5 (null hypothesis of no preference). Independent *t*-tests and two-way ANOVAs were performed to assess whether accuracy differed in the three experiments. Data were arcsine (square root)-transformed (Sokal & Rohlf, 1995). The mean ± SD are provided. Statistical tests were carried out using SPSS 18.0.

Results

Fish spent 59% of time in the two preference areas. No significant difference in accuracy was observed among the three ratios ($F(2, 27) = 1.01$, $p = 0.376$). Overall a significant preference was found for the trained numerosity ($t(29) = 3.60$, $p = 0.001$). When the three ratios were examined separately a significant result was found for ratios 0.25 ($t(9) = 4.13$, $p = 0.003$) and 0.5 ($t(9) = 2.33$, $p = 0.045$), but not for 0.67 ratio ($t(9) = 1.23$, $p = 0.250$, Fig. 39).

6.2.2. Experiment 2: Number only

This experiment investigated whether young guppies can learn to discriminate between two sets of dots when stimuli are controlled for the continuous quantities that co-vary with number and thus they can use only numerical information to solve the task.

Subjects, apparatus, and stimuli

Thirty 4-day-old fish were used as subjects. The procedure was the same described in Experiment 1.

As in Experiment 1 stimuli were black dots on a white background. There were the same three ratio conditions of Experiment 1 (n = 10 each; 1 vs. 4, 2 vs. 4, and 2 vs. 3 contrasts). Stimuli were controlled for the three most important continuous quantities that co-vary with number (Agrillo et al., 2009, 2010; Gómez-Laplaza & Gerlai, 2011b; Durgin, 1995): cumulative surface area, the density of elements and the overall space occupied by the arrays. Stimuli were controlled for continuous quantities using the procedure described in Section 4.1. In the stimuli used in the probe trials, cumulative surface area was equated to 100%.

Results

One fish in the condition 0.67 ratio was excluded during the training due to poor health.

Fish spent 57% of time in the two preference areas. No significant difference in accuracy was observed among the three ratios ($F(2, 26) = 1.21$, $p = 0.314$). Overall, no significant departure from chance level was found for the trained numerosity (one sample t-test, $t(28) = 0.32$, $p = 0.749$). When the three ratios were examined separately, no ratio was significant (0.25: $t(9) = 0.88$, $p = 0.404$; 0.50: $t(9) = 1.16$, $p = 0.275$; 0.67: $t(8) = 0.75$ $p = 0.476$).

Results of Experiments 1 and 2 were compared with a 2 x 3 ANOVA, with Stimulus (Number and continuous quantities/Number only) and Ratio (0.25/0.50/0.67) as between-subject factors. A main effect of Stimulus was found (*F*(1, 53) = 9.37, *p* = 0.003), no other factor was significant (Ratio, *F*(2, 53) = 2.09, *p* = 0.134; Interaction, *F*(2, 53) = 0.05, *p* = 0.949, Fig. 38).

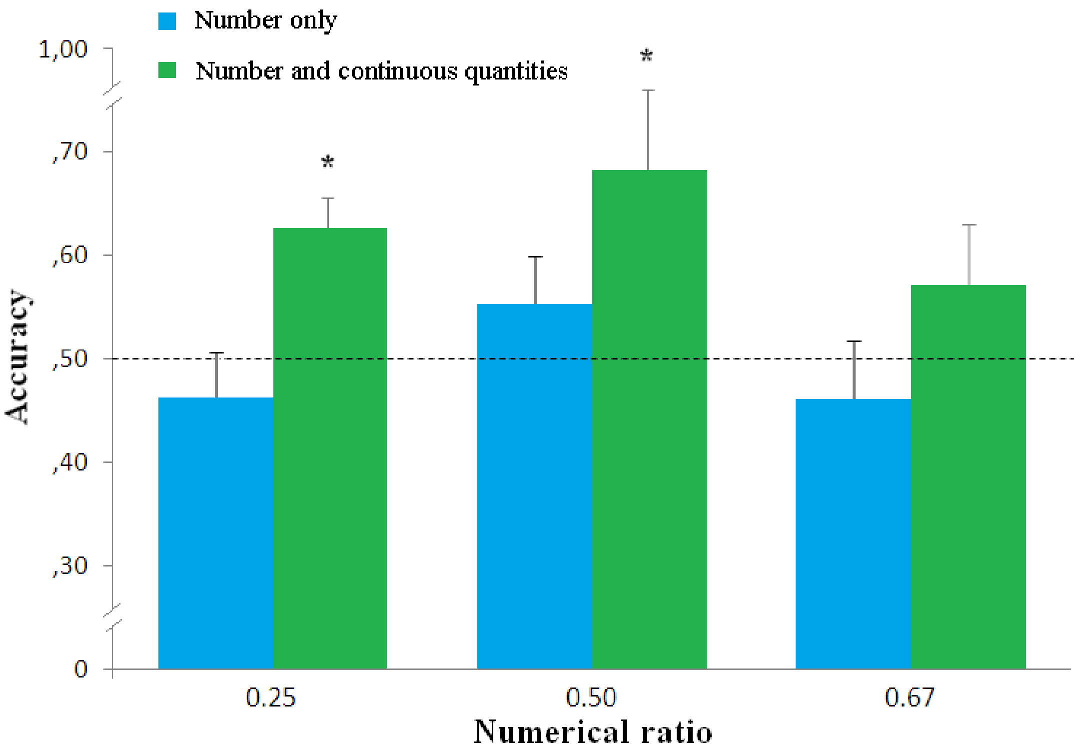


FIGURE 38: *Accuracy in discrimination of two quantities is plotted against numerical ratio when both number and continuous quantities were simultaneously available and when only numerical information was available. A significant preference for the reinforced numerosity was found only in the former condition and no ratio effect was observed. Asterisks denote a significant departure from chance level (P<0.05). Bars represent the standard error.*

6.2.3. Experiment 3: Continuous quantities only

Comparison of Experiment 1 and 2 indicates that fish were unable to discriminate quantities using solely numerical information, while they accomplished the task in the "number and continuous quantities" condition. There are two possible explanations for this finding. The first is that newborns are able to use continuous quantities but not able to represent numbers. Alternatively, as in Experiment 1 both number and continuous quantities were available, it is possible that a better performance might be due to the possibility of accessing multiple cues (Agrillo et al., 2011).

To unravel the question, in the third experiment, we tested fifteen young guppies under a condition in which stimuli differed in area but always maintaining a 0.5 ratio, while the numerical information was made irrelevant (3 vs. 3). As a reference, we compared the performance of fish with that of subjects of Experiment 1 and 2.

Results

Fish spent 53% of time in the two preference areas. Fish were unable to discriminate when only continuous quantities were available (0.53 ± 0.14, $t(14) = 0.84$, $p = 0.416$). This condition significantly differed from the condition of Experiment 1, either considering the whole sample or the sole elements of the 0.5 ratio ($t(43) = 2.1$, $p = 0.042$ and $t(23) = 2.1$, $p = 0.047$, respectively). No difference was found between "number only" condition of Experiment 2 and this experiment (whole sample, $t(42) = 0.82$, $p = 0.420$; ratio 0.5: $t(23) = 0.39$, $p = 0.698$).

6.2.4. Discussion

In Experiment 1 we found that 1-week-old guppies can be trained to discriminate between two sets containing different numbers of objects in just 20 reinforced trials. However, in the second experiment, when trained in the same condition using stimuli that have been paired for continuous quantities that co-vary with number (the cumulative surface area, the density of elements and the overall space occupied by the arrays), their performance dropped to chance level with all tested ratios. This suggests that young guppies can discriminate stimuli when they differ for both number and continuous quantities but they are unable to discriminate quantities using the sole numerical information. Adult guppies as well as several other fish species can easily learn a pure numerical discrimination even in experiments that adopted the procedure we have used here (Agrillo et al., 2011; see Section 4.1 and 4.2) The results of Experiment 2 indicate that this capacity is not inborn in guppies and must therefore appear after the second week of life.

The capacity to discriminate stimuli when non-numerical information, such as total area, is available but the failure when using the sole numerical information was previously reported for other species (infants: Feigenson et al., 2002b; cats: Pisa & Agrillo, 2009; dolphins: Kilian et al., 2003) and has been generally considered as evidence that numerical information is more complex to process than the continuous quantities that co-vary with number, such as area. Yet the results of the third experiment evidence that, for a newborn guppy, discriminating stimuli that differ solely for area is equally difficult to discriminating solely based on number, and that they can successfully discriminate between two sets only when both types of information are available. A somehow similar effect has been recently reported for numerical discrimination by adult fish that learned much faster to discriminate stimuli that differed for both number and area (Agrillo et al., 2011).

This phenomenon is probably not confined to fish. Redundant information is known to facilitate learning and memory in adult humans (Armelius & Armelius, 1974; Neil et al., 2006). As regards numerical abilities, multiple visual cues facilitated quantity estimation both in adults (Gebuis & Reynvoet, 2012) and infants (Iuculano et al., 2008).

In the natural world, numerosity co-varies with several non-numerical continuous quantities. The methods that are generally used in laboratory experiments to control for the non-numerical continuous quantities break down this natural association and may provide conflicting information that deteriorates the performance (Gebuis & Reynvoet, 2012).

The lack of discrimination in the contrast of 2 versus 3 when continuous quantities were available may appear surprising. However it is worth noting that quantity abilities increase in precision over development in human infants. As adult fish can discriminate 2 versus 3 even when the stimuli are controlled for continuous quantities (Agrillo et al., 2011), it is possible that also the quantity systems of guppies increase in precision over development.

The method we used here differs from a standard operant conditioning procedure. The capacity to discriminate was inferred from the time spent near the trained stimulus during final probe trials. This limits the possibility to obtain detailed information of the learning process instead examining individual learning curves in the standard procedure. However, with usual protocols, reaching a learning criterion on numerical discrimination normally requires several dozen or even hundreds of reinforced trials (Agrillo et al., 2009; Cantlon & Brannon, 2006), which is clearly ineffectual if the aim is to study age specific cognitive abilities in a fast growing species.

The results of this study largely differ from those obtained in guppies of similar age using the shoal choice paradigm (Bisazza et al., 2010). One-day-old fish were in fact observed to select the larger shoal for contrasts of 1 versus 2, 2 versus 3, and 3 versus 4 (but not 4 vs. 5, or 5 vs. 6). In that experiment there was no control of the continuous quantities and therefore newborns could have used these cues or a combination of continuous and numerical cues to identify the larger shoal. However in another experiment of the same study we showed that, at least for the 2 versus 3 contrast, newborns were able to select the larger group even when they could only see one stimulus fish at time, a procedure that makes extremely difficult or impossible to use continuous quantities such as the total area occupied by the shoal (Bisazza et al., 2010). Here young fish failed to discriminate between the controlled sets even when the discrimination task was very easy for a fish such as in the 1 versus 4 items. A comparison of the two studies thus seems to suggest that at birth guppies possess a sophisticated mechanism to enumerate and compare small groups of peers, but that this system is specific to the social domain and cannot be used to represent numbers in other contexts. This developmental dissociation speaks in favour of the existence of distinct quantification systems in fish that are characterized by domain and task specificity, each system operating largely independently from the others. The mechanism for comparing shoal size needs not to be specifically a numerical system. Specialized systems for processing biological stimuli have been often described in the literature, for example face-processing neural circuits in primates (Farah Wilson et al., 1998; Pascalis & Bachevalier, 1998) or systems for discrimination of biological motion in birds (Vallortigara et al., 2005). The innate ability to count peers may for example depend on a broader processing system for conspecific inputs which makes these information available also for estimating and comparing the size of social groups. Obviously, the possibility exists that item by item presentation used in previous study was less effective in controlling continuous quantities compared with the method used in the present study and further controls with the shoal choice paradigm are necessary, for example using computer animated stimuli, in which continuous quantities can be controlled in a way similar to that used in the present study.

From an evolutionary point of view the early appearance in fish of the ability to precisely estimate shoal size makes sense. The main tactics that fish have evolved to escape their predators is shoaling. In large aggregates, the risk of being captured by a predator is reduced (Krause & Ruxton, 2002). As guppies show no parental care, newborns are particularly vulnerable to predation and it is not difficult to envisage that selection has favoured a precocious development of an efficient system for assessing and comparing the size of social groups.

The use of numerical information to estimate the size of a social group could also be explained by the nature of the stimulus to be processed. Peers move incessantly in a three-dimensional space modifying their inter-individual distance. They frequently change orientation and hence the visible area, and can temporarily disappear behind solid objects or occlude each other. These characteristics of the stimuli may promote a system capable of keeping track of single items even when they change size and position or temporarily disappear from sight. This does not need to be specifically a numerical system. At birth, fish need to track live objects such as conspecifics, predators, and small live prey. They could thus be equipped with an object tracking system with characteristics similar to those described for humans (Trick & Pylyshyn, 1994). As a by-product of tracking multiple objects, this system could record their numerosity, although it is expected to allow handling a limited number of objects at once, around four in humans and non-human primates (Pylyshyn & Storm, 1988).

Further investigation is still required. For instance, there is an open debate as to whether non-human species (including fish) have two distinct numerical systems: a precise system for small numbers (1–4) and an approximate one for larger numbers (Agrillo, et al., 2012; Bonanni et al., 2011; Xu & Spelke, 2000). For a better comprehension of the quantification systems of young guppies, future studies comparing spontaneous choice tests and training procedure in the large number range (≥ 4) are also needed.

7. CONCLUSION

In the last decades, many studies have documented numerical abilities in non-human animals and increasing evidence suggests that the numerical systems of non-human animals may be the same as the pre-verbal numerical systems described in humans. In particular, the discovery in recent years that even simple organisms, such as fish, are capable of numerical abilities similar to primates has made possible the use of fish as an animal model to study numerical abilities in the absence of language.

However several questions about numerical capacities in fish remained unanswered and the present thesis aimed to fill this gap.

First of all, a novel procedure for training fish to discriminate between sets of stimuli differing in numerosity was set up as the previous methodology used to train fish was time-consuming, suitable only for social species and potentially stressful for fish (Section 4.1). The new procedure here described coped with these problems proving to be rapid, applicable to different fish species and efficient to study discrimination learning in fish in tasks requiring visual stimuli. As a consequence, the novel protocol was adopted in all the training studies here presented.

The question of whether all vertebrates share the same numerical systems, or rather numerical abilities have appeared multiple times during evolution in response to specific selective pressures imposed by the environment, represents one of the main issues of animal cognition. Despite the large amount of published data, mixed results have been reported in literature and the answer is still unclear. Part of the inconsistencies might be ascribed to the different methodologies adopted, making difficult any inter-specific comparison. Here the first inter-specific study using the same methodology in fish was presented (Section 4.2). When numerical abilities among five fish species as diverse as guppies, zebrafish, angelfish, redtail splitfin and Siamese fighting fish were compared, results showed interesting similarities opening the possibility of shared numerical systems among distantly related species. Hence, these results are more in accord with the existence of ancient quantification systems inherited from a common ancestor than with an independent evolution of numerical abilities in different species.

Another important question in the study of numerical cognition concerns the influence of contextual factors on the numerical capacities of a species. It is possible that the performance observed in a numerical task is limited to the specific context in which such abilities are observed rather than reflecting the full numerical competence of a species. This highlights the importance of using multiple experimental strategies before drawing firm conclusions about the ability of a species. To this purpose, Section 5.1 and Section 5.2 focused on the potential influence on fish numerical acuity of factors that normally occur in nature, namely the cooperative behavior within group and the perception of abstract items in motion.

Collective animal behavior, such as schooling fish or flocking birds, has attracted much attention as recent studies have provided evidence that group living can help to solve problems that are difficult or even impossible for single individuals. In the last decade, many studies have also shown that animals as diverse as mammals, birds and fish are capable of solving numerical tasks. However, all numerical cognition studies have tested subjects individually and it is not known whether collective behavior can enhance the capacity to solve numerical tasks in non-human animals. The comparison between the performance of individuals and dyads of guppies in two different numerical discrimination tasks showed that dyads performed better than singletons in selecting the larger group of social companions and also made better numerical discriminations of arrays of dots (Section 5.1). In addition, in both conditions, the better individual of the dyad spontaneously emerged as the leader. These results suggest a mechanism for the ecological advantage of schooling in fish: working together provides better information about shoal selection since individuals will follow leaders with better discrimination into larger shoals. Dyads performed better than singletons even when abstract stimuli were presented highlighting that collective behavior yields benefits that go beyond the single ecological context. Interestingly, the results here obtained aligned with data collected in adult humans where dyadic performance was superior than individual in a collective enumeration task (Baharami et al., 2013), thus suggesting that cooperation similarly increase numerical acuity in two distantly related species, such as humans and fish.

Another factor that might potentially affect numerical abilities is the motion of items. It is known that small and large number discrimination of groups of conspecifics in fish are differently affected by the quantity of movement whereas it is still unexplored whether fish can discriminate between two-dimensional figures in motion and whether the accuracy is the same in the small and large number range.

For example, it has been reported in adult humans that faster and more accurate estimation occurred when small numerosities of dynamic items were presented, supporting the hypothesis of two distinct numerical systems (Trick et al., 2003, Alston & Humphreys, 2004). A similar effect of items in motion has been here observed in fish: while a 3:4 ratio was not discriminated with static stimuli in either numerical range, guppies were able to discriminate this ratio with items in motion but only in the small number range (Section 5.2). To date, comparative psychologists disagree as to whether in non-human species a single system, the ANS, accounts for discrimination over the whole numerical range, or a distinct system, the OTS, operates over the small number range. Although the results do not represent a direct evidence for the existence of the OTS, the differential effect of motion reported in guppies reinforces the idea of separate cognitive systems for small and large numbers, in line with data collected in humans.

Despite no direct comparisons have been made between fish and humans in this thesis, the similarities between the two species are worth noting as they raise the intriguing possibility that the foundation of our numerical abilities might be evolutionarily more ancient than previously thought, dating back at least as far as the divergence between fish and land vertebrates, which occurred approximately 450 million years ago. Obviously this is just a speculation and more studies are needed to confirm this hypothesis.

The last part of the thesis focused on the development of numerical abilities using newborn guppies as a model species. Developmental studies can provide useful insights with respect to the existence of a single or multiple systems of numerical representation. For instance, exploring developmental trajectories of numerical skills in different contexts can help us to assess whether the same or distinct numerical systems are used in different tasks. Since an adequate method to study discrimination learning in newborn guppies was not available, the first step was to design a procedure by taking into account the social needs of young individuals in order to minimize potential stress due to social deprivation, without interfering with the normal development of their behavioral repertoire (Section 6.1). Newborn guppies were capable to learn a simple shape discrimination after few trials and the training method was then used to investigate their numerical competence using sets of two-dimensional objects, as commonly done with adult fish. The result that fish discriminated only very easy numerical contrasts in the range 1-4 when both number and continuous variables were available was in contrast with the results of shoal discrimination experiments (Bisazza et al., 2010) suggesting that newborns' capacity to use number is specific to social stimuli (Section 6.2).

On the whole, data on guppies, both adult fish and newborns, are suggestive of the existence of multiple quantification mechanisms in fish which are domain-specific and serve to solve a limited set of problems in accordance with the hypothesis proposed by different authors (Feigenson et al., 2004; Spelke, 2000)

The literature reported and the data collected in this thesis indicate that even fish, which are provided with a much smaller brain than warm-blooded vertebrates, can discriminate between quantities, process numerical information, and solve complex numerical tasks, in line with evidence in other research fields which suggest the possibility that processing numerical information might not require complex neural circuits. For example, a recent study using artificial neural networks (Hope et al., 2010) showed that fewer than 25-30 units may be sufficient for a system to represent numerosity, well below what traditionally believed until few years ago.

This goes together with recent discovery that bony fish possess several other cognitive abilities that were previously believed to be uniquely present in species provided with large, complex brains (i.e. mammalian and avian species). For instance, teleost fish have been shown to recognize up to forty familiar individuals, cooperate to achieve a common goal, learn new habits from experienced conspecifics, use tools, and have cultural traditions (reviewed in Bisazza, 2010; Bshary et al 2002; Brown & Laland, 2003). For all these reasons, fish may become a proper model to study cognitive abilities and in particular numerical competence.

Of course several topics need to be investigated yet. First of all, most of the studies on fish numerical abilities have investigated quantity discrimination in tasks where number and continuous quantities were simultaneously available. In this sense, further investigation is required to assess whether the capacity to use pure numerical information is restricted to a few species or instead, as advanced in the inter-specific study (Section 4.2), is a cognitive skill shared by all fish. Also, research should be enlarged to encompass a wider range of species. To date only near-shore freshwater teleost fish have been investigated, while no study has been done in saltwater and pelagic teleosts nor in cartilagineous fish. Only by collecting information on more species we will better understand the evolution of the cognitive systems underlying numerical abilities of fish.

It is worth noting that so far all fish studies have been restricted to the visual modality. In our own species, Tokita and colleagues (2013) reported a different performance in numerosity judgments tested in visual and auditory conditions, advancing the idea of multiple core number systems in which visual and auditory numerosities are mentally represented with different signal variabilities. The possibility exists that numerical acuity of fish is modality-dependent too. Future studies are needed to test this hypothesis. One possible way to investigate whether fish can elaborate numerical information in non-visual modalities could be testing blind fish that evolved for million of years in complete darkness, such as cavefish. Another possibilities is studying species that are active in both good and poor light conditions such as mormyrid fish to compare their numerical competence in the visual as well as in other sensory modalities. Partially related to this topic, also studies investigating cross-modal interaction of numerical information are needed, of the kind recently performed on primates (Jordan et al., 2005, 2008b). Again, no study investigated the relation between numerical and other magnitude abilities in fish. Several studies in humans (e.g., Agrillo & Piffer, 2012; Bueti & Walsh, 2009; Vicario, 2011) suggested the idea of a common magnitude system for non-symbolic estimation of time, space, and number, the so-called ‘A Theory Of Magnitude’ (ATOM, Walsh, 2003). Recent evidence supports the existence of a single magnitude system also in non-human primates (Haun et al., 2010; Mendez et al., 2011; Merritt et al., 2010). Studies investigating the validity of ATOM in distantly related species, such as fish, may help us to assess whether this common magnitude system is a recent evolutionary development of the primate lineage or rather is a common feature among vertebrates.

As a last note, the study of numerical abilities of fish might play a key role in the next future also to form a broader comprehension of the factors underlying the acquisition of our mathematical abilities. Recently, correlational studies (Furman & Rubinstein, 2012; Piazza et al., 2010) found that non-symbolic numerical systems are less accurate in individuals with deficit in the study of mathematics, which is in agreement with the idea that the construction of symbolic numbers depends on processes that are culture-dependent but nevertheless rooted in non-symbolic numerical systems (Halberda et al., 2008). Non-symbolic and symbolic numerical abilities were also found to be causally related in a study by Park and Brannon (2013).

As in other research fields, animal models may be fundamental in increasing our knowledge on human cognitive systems. For instance, the whole genome of zebrafish is already sequenced and several other species, guppies included, are currently being investigated. Considering the suggestion that vertebrates might share the same non-symbolic numerical systems (Beran, 2008a; Feigenson et al., 2004) and that dyscalculia might have a genetic origin (Butterworth & Laurillard 2010), the use of a model such as zebrafish might help us to understand the genetic origin of non-symbolic numerical abilities. In this sense, fish may help us not only to assess the evolutionary origin of non-symbolic numerical abilities, but also to better understand the foundation of our mathematical skills.

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